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Editorials

The Past and Potential

THE BODY of scientific knowledge relating to the cell might be compared to a field of grain at harvest time. The reapers who were first to enter the field made rapid headway and were often fortunate enough to reap a rich harvest. The gleaners who followed in the wake of the reapers expended much more time and effort and had to be content with a smaller yield. Of the last to come were the birds of the air who searched diligently for the few scattered grains which had escaped the reapers and gleaners.

The reapers of the past include Boreullus who as early as 1656 observed the microscopic constituents of animals and plants. The latter were designated as cells by Robert Hooke in 1665. Pertinent observations on the component units of living matter were also made by Lamark (1809), Dutochet (1824), von Mohl (1831) and others. The realization that cells were the fundamental biological units of all living matter is usually attributed to Schleiden (1838) and Schwann (1839) both of whom can be considered as successful reapers.

The work of Schwann established that animals and plants were made up of cells having both form and function. Their form received more attention than their function because morphology was a highly developed discipline of the times. Schultze (1861) confirmed the earlier work of Cohn (1848) and formulated what was later to be known as the protoplasmic theory. As a result the more primitive concepts of the past were abandoned and for the first time the cell was defined as a circumscribed mass of protoplasm surrounding the nucleus. Surviving to this day the definition has stood the test of time.

By the time the cellular theory of origin was accepted there was considerable knowledge of the macroscopic manifestations of disease. The realization that tissues were composed of cells led to the investigation of the role of the cell in disease. Such a relationship was considered by Goodsir (1845) and was further developed and expanded in Die Cellularpathologie of Virchow published in 1858. This work which comprised a series of lectures related the changes in disease to alterations in the cell.

Once the cell was established to be the basic unit of disease there was a rapid growth in scientific knowledge. Anatomists, biochemists, pathologists, physiologists, and zoologists attacked the problem and each made significant contributions. Depending on the discipline the cell was studied in its normal environment in the tissues or as an isolated unit. Such an approach was used in both basic and applied sciences. Studies of the cells in the blood stream developed rapidly and constituted a new discipline in medical science.

In addition to blood cells other cells occurring in the normal and abnormal secretions of the body were investigated. Of interest in this regard are the contributions of Donne (1838), Walsche (1843), Pouchet (1847), Donaldson (1853), and Beale (1860) and later work by Sanders (1864), Quincke (1875), Hamplyn (1876) and others. One of the more successful reapers in this field was the anatomist, George Papanicolaou, who recognized the importance of studies of the isolated cells. With the publication of his monograph on Diagnosis of Uterine Cancer by the Vaginal Smear, in 1943, a new avenue of investigation was opened and modern day Clinical Cytology was born.

There is still much to be harvested from the field of cellular knowledge. If the study of the cell, as we know it today, is to survive as a diagnostic procedure every attempt should be

made to utilize the full potential of the approach. This is not done when the method is used only to sort out those patients having abnormalities from those who do not. Such an application is like restricting the use of a fine Stradivarius to the renderings of an incompetent student of the violin.

The full potential of the method may be realized through continued research and by adapting the advances of other branches of medical science to the study of the cell. As with all microsampling technics great precision must be incorporated in the method if significant results are to be achieved. To correlate changes in cells with those occurring in the tissues, the chemical and functional properties of the cell must be investigated along with morphology. The cellular sample must also be evaluated independently since it may reflect properties or reactions which are not apparent in the tissues.

While investigation may be rewarding this alone will not make it possible for others to utilize the approach to its full potential. This can only be accomplished through research and teaching. One of these cannot be divorced from the other. Any information of value gained through research must be transmitted to others to be of use. Similarly, teaching is more fruitful when it is carried out in an atmosphere of research. With research and teaching, the full potential of the method may be realized and future harvests in the field of cellular knowledge may surpass any previously known.

JAMES W. REAGAN, M.D.

The Emergence of the Clinical Cytologist

SO RAPIDLY is progress being made these days that to keep abreast of only those advances in exfoliative and related cytology which are of clinical significance is no easy task. Indeed, to be well versed and experienced in all the aspects of cellular diagnosis which may be encountered in a large hospital or clinic requires the full time energy of a clinical scientist who can devote himself entirely to this specialty.

To qualify as a competent clinical cytologist special training is required. The number of disciplines and technics with which the clinical cytologist must be well familiar are rapidly growing.

The usefulness of any cellular examination is, of course, dependent first of all upon obtaining an adequate specimen. To do this properly requires attention to detail and in certain instances requires specialized or even elaborate methodology. In some instances it may be necessary for the clinical cytologist to collect these specimens himself, in others he must be available for consultation regarding the best technics for preparing specimens from various sites. Next, he must be experienced in reading cellular material collected from all parts of the body for the diagnosis of cancer and certain benign diseases, and this of course necessitates a familiarity with the histology and tissue pathology of these conditions as well. In addition to working with the routine well established staining methods the clinical cytologist must be prepared for working with special technics for specific purposes. Of particular interest at the moment, for example, are the evaluation of rapid fluorescent methods for cancer diagnosis which are being used in some laboratories, and also cytochemical staining methods for specific enzymes, steroids and other cellular constituents.

Nowadays the clinical cytologist must also be prepared to search for malignant cells in the blood stream. He must be an expert also on the effects of irradiation on tumor cells. The clinical cytologist must also be an expert in hormonal cytology and must therefore have a good working knowledge of the endocrinology of the female reproductive system. He must be familiar also with the cytology of the complications of pregnancy. There is every indication that soon the cytotechnologist will be intimately involved not only with the relative simple technics of nuclear sex determination, but the more elaborate, difficult but very exciting aspects of actual chromosomal studies for various diseases related to genetic defects.

As the demands on the cytology laboratory increase both in volume but especially in diversity of procedures, problems begin to arise. Who has the competence and time to supervise this expanding laboratory? Undoubtedly it should be in the hands of a physician who has the qualifications necessary for assuming the responsibility of making a diagnosis as important as cancer. Ideally this should be a pathologist, but only if the pathologist is sufficiently well versed in the entire field of clinical cytol-

ogy to assume such responsibility. In a few institutions there are now pathologists who are making cytology their specialty, and have become in the true sense of the word, clinical cytologists. There is a growing need for such people.

In other institutions the problem is being met by a cooperative effort of clinical scientists who are interested and have become expert in certain subdivisions of clinical cytology. This has worked out particularly well when such individuals have worked together as a team in a common cytology laboratory. This makes not only for communication among themselves of improved technics, methods and joint research projects, but also is very valuable in providing material from a variety of sources for cytotechnologists, students and pathology residents. Even in laboratories organized in this fashion it is apparent that an individual interested in cytology in all its aspects is necessary for the best coordination of the growing multi-phasic activities of the modern cytology laboratory. For this purpose there is bound to emerge a new specialist, the clinical cytologist.—ABRAHAM E. RAKOFF, M.D.

La Citologia Exfoliativa

LA CITOLOGÍA Exfoliativa ha llegado a ocupar, entre los diversos métodos de Laboratorio, un lugar privilegiado por su innegable utilidad en la detección del cáncer y por ser valioso auxiliar en el diagnóstico de ese padecimiento. Desde el estudio de las células vaginales (como lo idearon sus creadores) el método de Citología Exfoliativa se ha extendido para ser un procedimiento útil, en el estudio de células que se descaman prácticamente de cualquier cavidad del cuerpo. Debemos sin embargo acrecentar el valor del método citológico hasta lograr que los estudios celulares despejen las incógnitas que aún existen, siendo necesario para ello el estudio de las células en su doble aspecto estático y dinámico.

Los estudios citológicos no deben concretarse a observar células fijadas y teñidas; el citólogo no debe ser un morfólogo, sino que debe entender a la célula como una unidad funcional que respira, se nutre, excreta y se reproduce; debe comprender todos y cada uno de los mecanismos que éste elemento vital pone en juego para lograr verificar las funciones antes señaladas, y ser capaz de apreciar las modificaciones que éstas funciones sufren en los diversos procesos patológicos que necesariamente lesionan la integridad celular.

Para alcanzar ésto, habrá que considerar a las células fijadas y teñidas, como una mínima fracción en el estudio citológico y practicar observaciones microscópicas en células no alteradas por la fijación y la tinción tal como se logra por medio de la Microscopía de "Contraste de Fases." Habrá que hacer Cinefoto-Micrografía que nos permita comprender mejor los fenómenos de "Pinocitosis" y "Potocitosis," así como los intercambios nucleo-citoplasmáticos, los procesos degenerativos y las alteraciones concomitantes con la muerte celular.

El cultivo de células normales y patológicas permitirá repetir observaciones durante semanas, meses ó años. El empleo de sustancias marcadas aclararán muchas incógnitas en la vida celular.

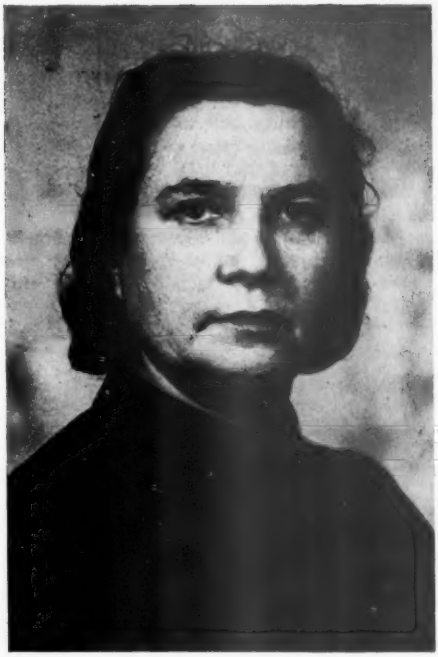
La Citoquímica hará que las coloraciones dejen de ser empíricas y ayudará a entender mejor las funciones de los Acidos Nucleicos base y principio de la vida celular.

La Microscopía Electrónica, la Microscopía en "Contraste de Fases," la Microscopía Fluorescente, el Cultivo de Células, la Citoquímica, y la Citogenética son poderosas armas que hoy tenemos; utilizándolas, se aclararán—muchas dudas que hoy persisten y la resultante será una Citología mas Científica. — DR. M. GONZÁLEZ RAMOS, *México, D.F., Mexico.*

In Memoriam

Maria Kawecka

1904-1960



MARIA KAWECKA was born in Przemyśl, Poland on November 6, 1904. After graduation from Przemyśl College she attended the King's Jan Kazimierz University in Lwów where, in 1930, she received her medical degree. Her internship and residency was completed at the University Hospital in Lwów and she then returned to Przemyśl to practice gynecology in the Gynecological and Obstetrical Ward. During World War II both her husband and brother were killed and Dr. Kawecka remained in hiding in Warsaw. In 1945 she went to Gliwice where, in 1950, she joined the staff of the Institute of Oncology. Two years later she began her work in the Oncological Out-patient Department. During this period she organized the Cytological Laboratory which became distinguished for the high quality of its scientific work.

Dr. Kawecka realized the importance of early detection of uterine carcinoma and is known as one of the first in Poland to employ exfoliative cytology as a diagnostic technic. She was also the first author to publish a manual dealing with a complete understanding of the problems in this field.

Dr. Kawecka trained many doctors in cytodiagnosis, both individually and in special courses organized under her direction. This made possible the organization of the Outpatient Department in Silesia and in many other parts of the country. In addition, many prophylactic mass surveys in Silesia were executed due to her initiative.

Dr. Kawecka also trained doctors in bronchial cytology, did research on sources of diagnostic error in bronchial cytology and applied cytology to the diagnosis of recurrence of cancer after radiation-therapy, but most of all, Dr. Kawecka was interested in the diagnosis of carcinoma *in situ* of the uterine cervix. She firmly believed electroconization as therapy was sufficient and had the advantage of allowing the woman to bear more children.

Her research, practice, and training programs did not prevent her from being an active and contributing member of the Inter-Society Cytology Council, the Board of the Polish Gynecological Association in Silesia, the Polish Oncological Association, and the Polish Tuberculosis Association. She was invited to the International Symposium on Exfoliative Cytology in Brussels, and wrote several articles on carcinoma *in situ* which appeared in ACTA CYTOLOGICA. Among her 27 major publications are many dealing with the problem carcinoma *in situ* and cancer detection.

Dr. Kawecka was an esteemed friend to her younger colleagues and students. She was a strict teacher—not only with her students but with herself in her own clinical and scientific contributions. Dr. Kawecka has been decorated with the High Polish Orders. Her untimely death on September 13, 1960 interrupted her creative and energetic life just as she was about to become a Professorial Lecturer (Docent) in Gynecology. We shall all hold her teachings in the highest regard and continually remember her for her unswerving devotion in the interest of her patients and her students.

JADWIGA BYLINA
HANNA STARKIEWICZ

Cytology of Voided Urine with Special Reference to "Benign" Papilloma and Some of the Problems Encountered in the Preparation of the Smears

JOHN GEOFFREY SANDISON CRABBE*

London, England

ONE of the most worthwhile uses of exfoliative cytology, outside the gynecological and respiratory fields, is the routine examination of urine sediment for the control of occupational bladder tumors, a fact which does not seem to be fully appreciated in cytological circles. When dealing with a population which has been exposed in the past to bladder carcinogens and who are, therefore, liable to develop bladder tumors at some time, it is absolutely essential that all possible means should be adopted for detecting these growths at as early a stage as possible. Since its introduction as a diagnostic measure for this purpose this is precisely what the Papanicolaou technic does help to do and, in so doing, it has already, in our experience, helped to prolong the lives, considerably, of most of the men who have been affected. For the past nine years in Great Britain the method has been used in the Dyestuffs Industry[5] and, latterly, in the Rubber Industry and although similar work has been carried out in America, Germany[10] and Italy for some years, no results have been published from these countries until last year when a preliminary report was made at the 7th Annual Meeting of the Inter Society Cytology Council by Melamed, Koss and Ricci[16] on the control of some 280 men exposed to para-amino diphenyl, a powerful bladder carcinogen.

Apart from the routine screening of the process workers the cytological technic has a great advantage over other methods of control in that it can be used in these industries as a frequent check on those groups of workers whose exposure to the carcinogens may have been slight and, therefore, whose chance of tumor development may only be remote. Furthermore it can be used in pre-employment examinations of new workmen and lead to the elimination of persons being taken on with unsuspected tumors.

There are, also, other possible ways in which the method could be utilized within these industries. It could be used as a means of screening the urines of workmen exposed to any products which may, in the future, fall under suspicion. With the ever-increasing range of new organic chemicals, this aspect is very important. It could also be used as one of the earliest means of assessing the real protective value in plant design or process.

In contrast to this success with the method when applied to the control of occupational bladder tumors, Solomon, Hyman, Amelar and Europa[23] state that urinary studies in exfoliative cytology have failed to secure a niche in the diagnostic field of cancer of the urinary tract which is further borne out by the experiences of Presti and Weyrauch[18] and Dimette, Sproat and Klimt[8] in

* From the Department of Pathology, The Royal Free Hospital, and Consultant Cytologist, I.C.I. J. G. S. Crabbe, M.R.C.S., L.R.C.P., is Lecturer in Cytology, Royal Free Hospital School of Medicine, Honorary Assistant Clinical Pathologist, Royal Free Hospital, and Consultant Cytologist, I.C.I. Ltd., The Frythe, Welwyn, Herts.

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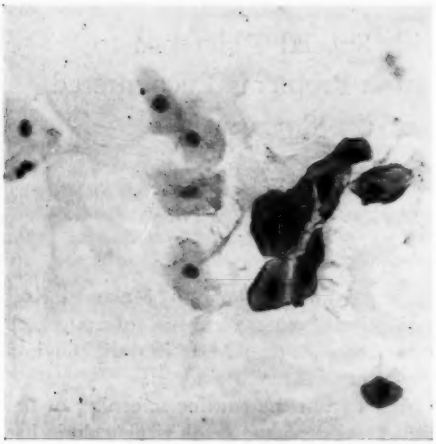

FIG. 1. A smear of urine sediment from Mr. A. showing normal squamous and transitional epithelial cells (from x400).

cancer detection clinics and surveys among army veterans. There are several reasons for this. Although malignant tumors of the bladder, ureters and renal pelves are fairly easy to detect by the method, it is less successful with the detection of papillomata. Then tumors of renal parenchyma usually do not exfoliate their cells into the urine until the tumor is far advanced and has invaded the renal pelvis, and even then the cellular picture is difficult to interpret. Also cells from prostatic carcinomata are seldom found in ordinary voided urine unless massage has first been performed. Finally, one of the main reasons is because it is often only possible to examine random specimens. In the control of occupational bladder tumors, on the other hand, the urine of each man is examined repeatedly three or four times a year and, if any of the specimens are unsatisfactory, further samples can be requested. Foot, Papanicolaou, Holmquist and Seybolt[11] state that the value of repeat specimens cannot be disputed. The whole success of urinary cytology depends upon the repeated examination of several different samples thus ensuring that some of them will have adequate cellular content. The first early morning specimen is probably the best, as it is more likely to contain a greater concentration of cells than that from a bladder that has been well washed out by imbibed fluids. This is not always obtainable, however, particularly from industrial workers and it does not matter very much provided the urine is examined often enough. The repeated examination of urine is of particularly great importance when endeavoring to detect the presence of the "benign" papillomata, tumors which seem to exfoliate their cells only very sporadically in contrast to the definitely malignant growths which usually shed their cells freely.

Normal Cytology of Transitional Epithelium

In the control of occupational bladder tumors it is almost entirely men who have been at risk, but during the last war in Great Britain a few women were also exposed to the hazard. The majority of urines examined, therefore, came from men. Normal male urine when processed by the

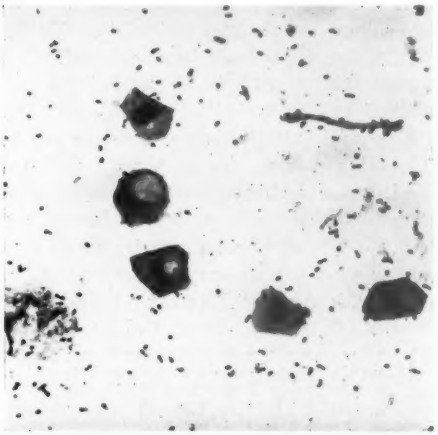

FIG. 2. A smear of urine sediment from Mr. B. showing normal bi-nucleated and vacuolated transitional epithelial cells. This specimen was rather stale and contains many yeast-like fungi (from x400).

ordinary Papanicolaou method contains very few cells but these are usually sufficient to be able to tell that the deposit has not washed off and they are usually accompanied by a faintly staining well defined background of amorphous phosphates or other crystals. The smears of voided urine from women, of course, contain many squamous cells from the vulva, but these do not interfere unduly with the reading of the slides provided one knows that the urine is that of a female.

The normal cells in urine arise, mostly from the transitional epithelium of the urinary tract and these vary greatly in size, shape and appearance generally. Bi- and multi-nucleation is common and there may be marked vacuolation of the cytoplasm. It is this variation in the morphology of exfoliated transitional epithelial cells which is responsible for some of the difficulties in the interpretation of urinary sediment smears, and which can lead astray persons inexperienced in this branch of cytology. The cell picture in normal urine differs greatly from individual to individual, so much so that it is almost possible at times to recognize a particular person by the cells in his urine (Fig. 1-3). It is only in disease of the urinary tract that blood, pus and debris are present in quantity.

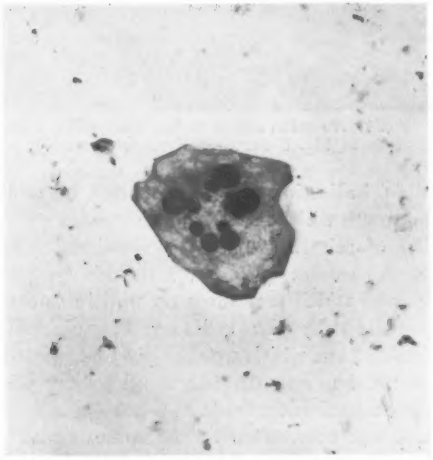

FIG. 3. A smear of urine sediment from Mr. C. showing a large multinucleated transitional epithelial cell, which is quite normal. This man always sheds a fair number of cells of this type into his urine (from x400).

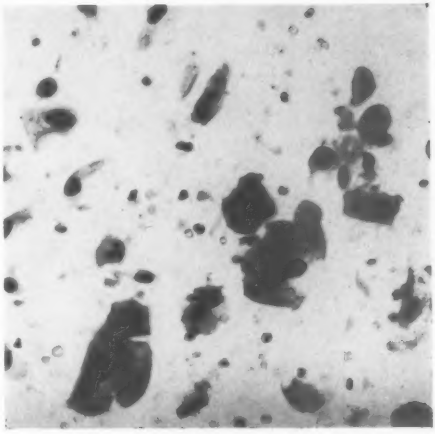

FIG. 4. Malignant cells in urine sediment (from x400).

Detection of "Benign" Papillomata

It is generally agreed by most workers in this field that exfoliative cytology is of great value in detecting malignant growths (Fig. 4) of the bladder but they are more dubious about its ability to detect the simple histologically benign papillomata.[4, 6, 13, 14, 21] However, it is just as important to detect these tumors at as early a stage as possible as it is the frankly malignant ones, because some of them behave clinically like malignant tumors even though their structure may be histologically benign. Dr. Joseph Bamforth (personal communication), Pathologist to Imperial Cancer Research possesses a beautiful section of a simple papilloma of the bladder which shows emboli of papilloma cells blocking some of the submucosal blood vessels. Indeed there is much controversy about the innocence or otherwise of the simple papilloma. Cuthbert Dukes[9] states that a tumor should only be called benign if the covering epi-

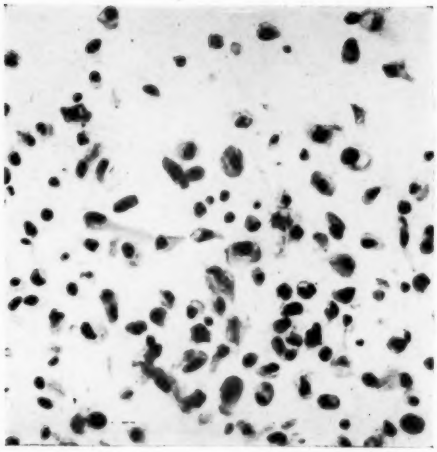

FIG. 5. Many transitional epithelial cells in a smear of urine sediment from a patient with a "benign" papilloma (from x400).

thelium closely resembles normal transitional epithelium as seen in healthy bladder mucosa, and Deming[7] accepts the papilloma with strictly benign histological features as being benign. On the other hand Marshall[15] points out that among patients who had simple benign papillomata, proved on adequate biopsy, about one in six are liable to develop proved carcinoma in five years time. Broders[3] and Ash[1] consider them all to be malignant to some degree and class them as grade one carcinomata.

It is not surprising then that there is some degree of uncertainty as to the features of the cells exfoliated from such tumors. The characteristics will depend on the structure of the growth itself. If the covering epithelium of the tumor closely resembles normal transitional epithelium then it is unlikely that the cells shed will deviate from the normal sufficiently to make a definite diagnosis. The only possible indication to the presence of such a growth will be an abnormally large number of relatively normal transitional cells in the smears (Fig. 5). It is this type of growth which may show up well in smears prepared by the ordinary Papanicolaou method but which would only be confused or not be demonstrated at all by the cell concentration methods.

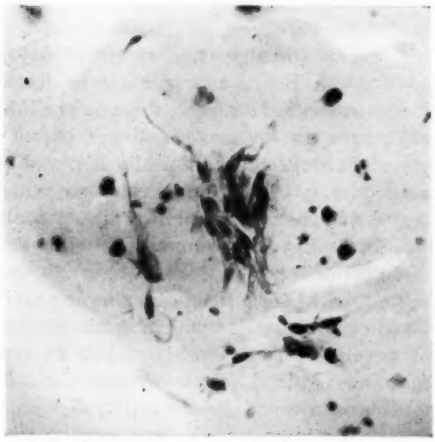

FIG. 6. Atypical cells in a smear of urine sediment from a patient with a "benign" papilloma (from x400).

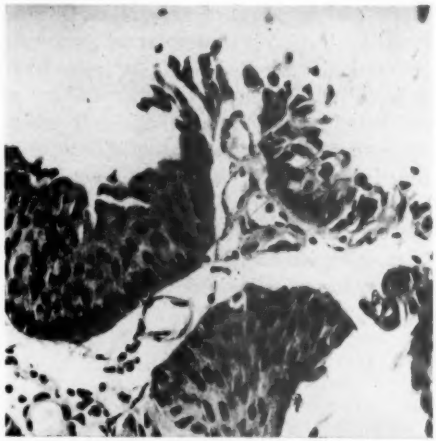

FIG. 7. A section of the tumor from which the cells in Fig. 6 were exfoliated (from x400).

If the epithelium of the tumor consists of more atypical cells such as cuboidal cells and these cells are visible in the urine then it becomes much easier to detect the presence of such a growth (Fig. 6, 7). These

atypical cells show none of the criteria of malignancy but they are quite different to normal transitional cells.

The characteristics of smears showing cells exfoliated from benign papillomata can be described as follows:

1. The cells may be normal transitional cells but present in very large numbers, sometimes associated with many red blood cells.
2. The cells may be small and atypical, of the nonciliated cuboidal or columnar type or they may be elongated or fusiform in shape and all approximately the same size.
3. Their nuclei are small and round, oval or slightly elongated in shape and approximately equal in size.
4. The chromatin of the nucleus is usually normal in appearance though there may be some hyperchromasia or a fairly pronounced nuclear border.
5. The smears may contain such cells singly and in large numbers or groups and clusters (Fig. 8, 9) may be present, sometimes showing a pallisade formation. The clusters may consist of small balls of cells or of cells with nuclei of equal size but with little or no cytoplasm visible. Sometimes fairly large sheets of cells may be seen suggesting that small fronds of tissue have actually been shed.

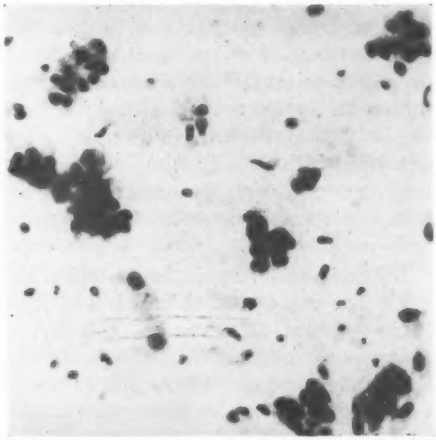

FIG. 8. Groups and clusters of cells in urine sediment from a case of "benign" papilloma (from x400).

FIG. 9. A small sheet of cells in urine sediment from a case of "benign" papilloma (from x400).

However, cells with rather similar characteristics can arise from other parts of the normal genito-urinary tract, particularly in the male. The epithelium lining the spaces, tubules and ducts of the prostate can give rise to nonciliated cuboidal and columnar cells. Also, in both sexes similar cells can

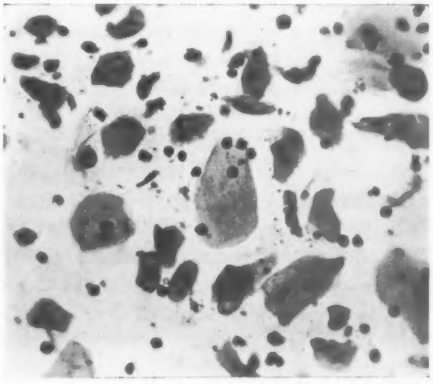

FIG. 10. A smear of normal urine sediment prepared by a cell concentration method showing many transitional epithelial cells, erythrocytes and leukocytes (from x400).

be shed from the epithelium lining the lacunae of Morgagni and the glands of Littre. When cells of this type are seen in urine they can be very misleading. However, they are usually smaller than papilloma cells and more feathery in appearance and almost always associated with streaks of mucus.

In reviewing the new tumor cases detected among Dyestuffs workers since the method was first introduced it was found that the diagnostic accuracy of the cytological detection of simple papilloma was not nearly as accurate as that of carcinoma. Among the 80 new tumor cases found, 37 tumors were shown to be simple papillomata on biopsy yet only 11 of these were diagnosed as such by the smears. Six of these 37 cases showed no abnormal epithelial cells at all (False Negatives) and were detected because of increasing microscopic hematuria. It is interesting to note that the remaining 20 cases all had frankly malignant smears.

Because of the difficulties associated with the detection of papillomata by the Papanicolaou method alone, it becomes necessary in the control of occupational bladder tumors to utilize also the test for the presence of microscopic hematuria in order to ensure that as many tumors as possible can be detected at a stage when they are easily amenable to treatment and long before they give rise to symptoms.

Problems Encountered in the Preparation of Smears

At the Sixth Annual Meeting of the Inter-Society Cytology Council in November 1958, Solomon, Hyman, Amelar and Europa[23] reported on some of the problems which confronted them when dealing with urinary cytology and the methods which they adopted to overcome them. Similar problems arose during the routine screening of some 3,000 workmen in the Dyestuffs and Rubber Industries.

1. Collection of Urine: This involves very little trouble in hospitals and cancer detection clinics, but when it comes to collecting specimens from apparently healthy workmen, this is by no means so easy. The difficulties occur chiefly in big factories covering a large area of ground where the men may be working all over the place and on different times schedules. In these cases the supervision of the urine collection is difficult and irregularities are apt to occur. However, in one small factory it has been possible for the men to come up to the medical center to pass their urine into specially numbered jars or for a medical orderly to go around the site to collect the individual specimens. Finally, in one of the industries concerned, a group of small factories which are distributed all over the country send their specimens through the post to a central laboratory for processing and interpretation.

2. Preservation: Again in a hospital the urine can usually be collected fresh and then processed at once, but this is not always possible in routine factory examinations and it was one of the first problems encountered when this work was started. This applied particularly to men on shift work where the specimens may lie around overnight or even over the weekend.

It was therefore necessary to utilize some method of fixation and preservation of the cell contents of the urine.

It has been stated[20] that urine must be processed within an hour of micturition otherwise the cells in it will deteriorate so rapidly that interpretation becomes impossible. In point of fact, urine samples known to contain cells of all types including tumor cells, have been left, some with and some without any fixative in them, in an atmosphere well above room temperature for up to three days before they were processed. In all of them, even the ones that contained no fixative and smelled very unpleasant, easily identifiable cells of all types including

tumor cells, red and white blood cells and epithelial cells were visible. These particular specimens were, of course, grossly contaminated by fungi and bacteria and contained many obviously degenerated cells. While it must be emphasized that it is essential to obtain the specimens as fresh as possible, this serves to demonstrate that cellular degeneration does not proceed so rapidly as one often supposes.

In order to preserve the cells a variety of reagents were tested. Ruth Graham[12] suggests that the addition of 10 cc. of formalin to 90 cc. of urine will preserve it but it was found that after some hours the deposit became useless for processing or the resultant smears were quite unsatisfactory. Other types of chemicals such as acetone, chloroform and other formalin containing fixatives were equally useless and so also were toluene, benzene and the mercurial salts and their various combinations with other reagents.

Papanicolaou[17] recommends the addition of a roughly equal amount of 95 per cent alcohol to the urine and this was undoubtedly found to be the best fixative and preservative. It was shown that the addition of even only a relatively small proportion of full strength industrial methylated spirit to the urine was sufficient to preserve the cellular contents for several days without the specimen having to be stored in a refrigerator.

3. Transport: For the purposes of transport the urine is passed into a wide mouthed polythene jar with a screw top and of about 4 oz. capacity into which is put about 1 oz. of industrial alcohol. This can then be safely sent through the post.

4. Processing: After a brief early period of trial and error no further difficulty was found in making the centrifuged deposit adhere to plain microscope slides if certain simple precautions are taken and the staining method for urine smears recommended by Papanicolaou[17] is followed. Of all the thousands of smears which have been made in the laboratories since 1951 it is very seldom that any of them washed off during fixation or processing.

The use of frosted glass slides was of no advantage whatever. The method employed for making the smears stick to the slides is as follows. The slide is first coated with a film of glycerine albumen and a portion of the deposit smeared upon it with the aid of another slide. This is then left in the air for a time to allow it to adhere properly. This takes quite a few minutes, though it varies from specimen to specimen, and then, when it starts to show signs of beginning to dry such as clouding of the edges and thinner parts of the smear, it is immersed in the ether alcohol fixative, where it remains until it is ready for staining. The whole point of the operation is that the smears must not be put into the fixative too soon, but on the other hand they must not be allowed to dry. The celloidin technic can be used as an additional safeguard to prevent the smears washing off but it is not absolutely necessary and it is certainly not used in the factory laboratories.

5. Concentration of Cellular Contents: Owing to the scanty number of cells in urine smears a number of cytologists[6, 19, 22, 23] have tried out various methods to concentrate all the cells in a given specimen in order to increase the accuracy of diagnosis and also cut down the time in reading the slides. Some of these methods have been tried out for routine work, but so far it was found that generally, the disadvantages outweigh the advantages. In the first place the methods of preparation are usually too cumbersome for a busy laboratory processing many specimens a day and with some of the methods, the number of samples that can be prepared at one time is limited. Furthermore, the urines must be fresh and contain no preservatives. The final preparations, though often very beautiful (Fig. 10), contain such a concentration of cells

that little time is saved in the screening and in urines which contain malignant cells there were no advantages over the ordinary Papanicolaou method. The chief disadvantage of these concentration methods was that they completely confused the interpretation of those smears containing cells exfoliated from certain types of histologically benign papillomata.

In one of the smaller factories a modification of Rofe's method has been tried out with some success[2] and more recently another laboratory has been testing one of the Millipore filter methods, but it is too early yet to be able to state whether it has any advantages for routine work of this sort.

It is rather interesting to note that in the urines taken from normal healthy subjects and prepared by one of these cell concentration methods[19] that the smears of nearly all of them contained an appreciable number of red blood cells.

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Cytologic Study of Bone Marrow Cells by the Papanicolaou Method

JONAS VALAITIS, M.D., ELIZABETH A. MCGREW, M.D.

From the Departments of Pathology, West Side Veterans Administration Hospital and University of Illinois College of Medicine, Chicago, Illinois

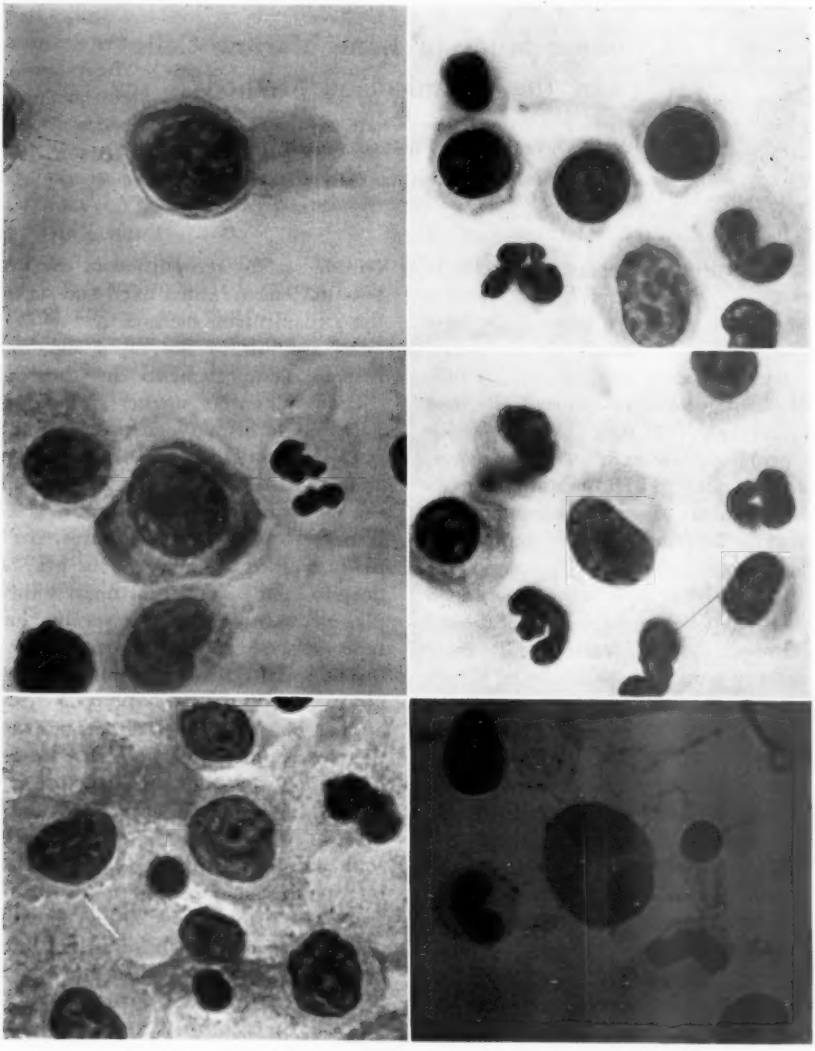
IN the identification of cancer cells in concentrates of peripheral blood,[3] normal and abnormal immature blood cells have sometimes been mistaken for malignant cells. Megakaryocytes are particularly difficult to distinguish from cancer cells, and small malignant cells may closely resemble other hematopoietic cells in size, nuclear-cytoplasmic ratio and chromatin characteristics. Immature marrow elements are found more frequently in the highly concentrated cellular samples prepared for tumor cell isolation than in the usual blood or buffy coat hematologic preparations.[1] Furthermore, they are undoubtedly more numerous in patients with advanced cancer than in normal individuals, especially if the patients are receiving radiation or chemotherapy. In an effort to learn to recognize these hematopoietic cells in Papanicolaou preparations, a comparative study of bone marrow cells was undertaken.

Sixty-three bone marrow preparations from patients with various hematologic disorders were studied by the Papanicolaou technic[2] as well as by the usual Wright-Giemsa method. Marrow aspirated from the sternum or iliac crest was spread on slides, one of which was fixed immediately in ether-alcohol solution and stained by the Papanicolaou method and the other fixed in methyl alcohol and stained with the Wright-Giemsa stain. By studying the paired slides it was possible to establish criteria for the recognition of the hematopoietic elements when fixed and stained by the Papanicolaou method.

As a result of the rapid fixation, the hematopoietic elements in the Papanicolaou slides were smaller and more compact than in hematologic preparations. The nuclear chromatin was more deeply stained and its pattern more distinct. Except for the megakaryocytes, the marrow cells had scanty cytoplasm. In all forms, the cytoplasm stained a homogeneous gray-tan or deep to light blue. Unfortunately the cytoplasmic staining was not specific for cell type or stage of development.

The usual classification of nucleated red blood cells into basophilic, polychromatic and oxyphilic normoblasts was not possible therefore, and it was necessary to depend largely on the size and structure of the nucleus, as indicated by Whitby and Britton,[4] in identification of these cells. They could be divided into pronormoblasts and intermediate and late normoblasts in this way. The pronormoblasts had finely granular nuclear chromatin, indistinct blue nucleoli, a somewhat beaded nuclear membrane and a small amount of usually basophilic cytoplasm with a pale perinuclear halo (Fig. 1). The chromatin of the early normoblasts was similar and an occasional nucleolus was seen. The intermediate normoblasts (Fig. 2) showed a coarse, lumpy, somewhat radially arranged chromatin, and the nuclear membrane was discontinuous. The nucleus was centrally located and the cytoplasm was not thickened at the periphery. The late normoblasts were similar ex-

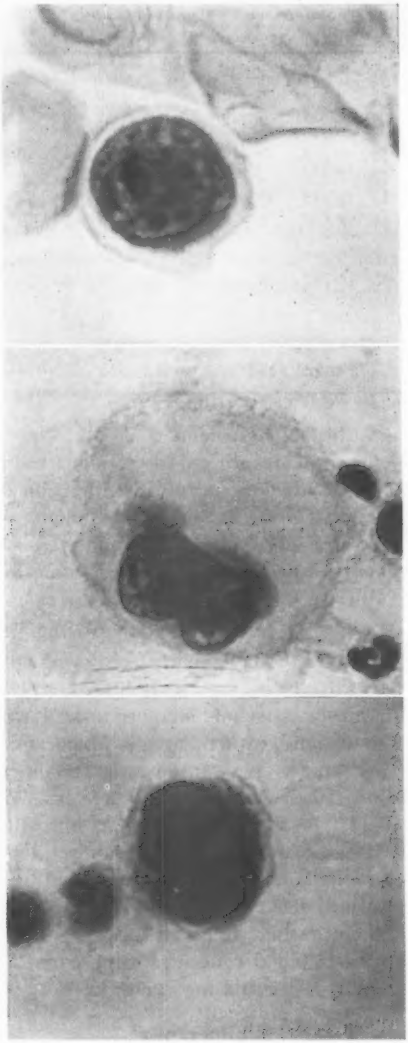
Presented at the Eighth Annual Meeting of the Inter-Society Cytology Council, 1960, Chicago, Illinois, U.S.A.



FIGURES 1-6. Bone marrow cells stained by the Papanicolaou Method.
1. Pronormoblast. 2. Intermediate normoblast. 3. Early megaloblast. 4. Metamyelocyte.
5. Monoblast. 6. Binucleated plasma cell.

cept that the nucleus was small and composed of dense structureless chromatin. The pronormoblasts averaged about 15 microns in diameter, the early normoblasts 12 microns, the intermediate normoblasts 10 microns and the late normoblasts 7 microns.

Megaloblastic cells from patients with pernicious anemia showed characteristic nuclear features. The nuclei of the promegaloblasts displayed fine chromatin granularity and large blue nucleoli. The early megaloblasts had similar chromatin with focal



FIGURES 7-9.
7. Plasmablast. 8. Megakaryocyte. 9. Megakaryoblast.

areas of chromatin dissolution and irregular large blue or occasionally red stained nucleoli (Fig. 3). These latter nucleoli were similar to those observed in the myeloblasts of acute leukemia. The average diameter of the promegaloblasts and early megaloblasts was 20 microns.

The early stages of the myelocytic series were characterized by a heavy, distinct nuclear membrane. The promyelocytes in addition had fine chromatin granularity with an occasional blue stained nucleolus. The neutrophilic myelocyte also had fine nuclear chromatin and its nuclear membrane was less distinct. The nuclei were large, round and somewhat eccentrically located. The cytoplasm of these cells was neutrophilic and the azurophilic progranulations usually seen in Wright-Giemsa slides were not visible. The nuclear chromatin was increasingly coarse in the metamyelocytes, bands and segmented neutrophils. Many of the metamyelocytes (Fig. 4) displayed an oval rather than round configuration, often without obvious indentation of the nucleus. The specific eosinophilic granules of the eosinophils did not stain as well as in Wright-Giemsa preparations, and no basophils were identified in the smears stained by the Papanicolaou method. The neutrophilic promyelocytes averaged 20 microns in diameter, the myelocytes 16 microns and the metamyelocytes 12 microns. The myeloblasts studied were from a case of acute myelocytic leukemia. These had distinct nuclear membranes and coarse chromatin with large, irregular, usually red stained nucleoli. These cells averaged 20 microns in diameter.

Monocytes were seen infrequently in this material and definite criteria for their identification have not been established. A case of acute monocytic leukemia furnished numerous monoblasts (Fig. 5), however. These had distinct coarse intranuclear chromatin with a moderately heavy nuclear membrane and large multiple red stained nucleoli. They measured about 20 microns in diameter.

Identification of mature lymphocytes was not difficult although some, with small patches of incomplete chromatolysis, resembled late normoblasts. Prolymphocytes had slightly clumped chromatin and indis-

TABLE 1. *Differential Counts of Bone Marrow Smears Stained with Papanicolaou and Wright Stains (10 cases)*

	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W
Promyelocytes	0	0	1	1	1	1	1	2	1	2	0	1	0	2	0	0	0	1	0	1
Myelocytes	2	3	7	8	1	1	4	5	2	4	3	5	4	9	3	5	5	4	2	7
Metamyelocytes	9	8	12	14	3	9	10	12	8	15	7	8	18	16	9	9	13	7.	10	14
Bands	6	5	7	10	8	8	5	7	5	10	7	6	7	7	6	9	9	15	9	11
Segmented n.	17	12	31	34	18	17	13	9	13	14	21	12	18	13	20	15	24	33	38	16
Eosinophiles	1	1	1	1	2	2	1	1	1	1	2	0	1	8	4	9	1	4	0	0
Pronormoblasts	0	1	0	0	0	0	1	1	2	2	0	0	0	0	1	1	0	0	0	0
Normoblasts	13	14	23	13	21	29	43	41	35	20	13	10	15	8	30	28	19	16	24	25
Lymphocytes	13	31	7	7	18	19	7	5	17	13	36	38	15	12	15	9	7	3	3	4
Monocytes	0	0	0	1	2	2	1	0	0	0	2	3	2	3	0	0	1	1	1	2
Plasma cells	28	16	3	2	2	2	1	1	5	5	0	2	7	7	3	3	7	7	4	4
Autolytic cells	11	9	8	9	9	10	13	16	13	20	9	16	13	15	9	12	14	9	9	16

tinct blue stained nucleoli. They measured about 12 microns in diameter.

Plasma cells were characterized by an eccentric location of the nucleus and a clear paranuclear "hof." The nucleus was densely stained but several irregular pale areas could usually be made out. Binucleated forms were not uncommon (Fig. 6). Plasmablasts from a case of multiple myeloma (Fig. 7) showed large round nuclei, often centrally located, with fine distinct chromatin granularity and prominent red stained nucleoli. These cells averaged about 15 microns in diameter.

Mature megakaryocytes had multiple lobulated nuclei with distinct nuclear outlines, a few coarse chromatin granules and multiple small basophilic nucleoli arranged in symmetrical lobules, with a distinct perinucleolar achromatic zone. In younger forms (Fig. 8), a few large block-like nuclear lobes contained dense chromatin in irregular streaks, but here also, small nucleoli were set off from the adjacent chromatin by small round clear zones. The megakaryoblasts often contained two "mirror image" large dense nuclei with rather more finely granular chromatin but without the characteristic nucleolar arrangement (Fig. 9). Naked nuclei of all stages showed similar structure.

Differential counts of 500 cells were made from the Papanicolaou and the Wright-Giemsa smears of ten cases. The results indicated that accurate identification of most cells can be made, although the figures for normoblasts, lymphocytes, and segmented neutrophils differed somewhat (Table 1).

Summary

The morphologic characteristics of bone marrow cells in smears prepared by the Papanicolaou technic were compared with those in standard hematologic preparations of the same marrow in 63 cases. Some criteria for their identification have been described. It is important that immature hematopoietic cells be accurately identified in Papanicolaou preparations and distinguished from small tumor cells, because of their rather frequent appearance in peripheral blood concentrates prepared for the study of circulating cancer cells.

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Cytologic Diagnosis of Carcinoma of the Urinary Bladder by Fluorescence Microscopy

W. O. Umiker, M.D.*

Ann Arbor, Michigan, U.S.A.

Fluorescence microscopy has provided a useful method for the detection of malignant cells in urine sediment, as well as in other body fluids.[3, 4] The acridine-orange staining procedure of Bertalanffy[1] is simple and rapid. Morphologic details are precise, and carcinoma cells stand out in sharp relief against the dark background. This report of additional observations on its application to urinary exfoliative cytology substantiates preliminary conclusions, and highlights its merits and limitations relative to standard light microscopy.

Materials and Method

Urine from patients under study for hematuria was processed according to the technic of Papanicolaou.[2] The quantity of urine varied from 50 to 200 ml. per specimen. Maximum adherence of the sediment to the slides was effected by (1) adding a drop of albumin to the sediment in the centrifuge tube, (2) coating the microscopic slides with a thin layer of albumin, and (3) coating the smears with 0.5 per cent celloidin in 95 per cent ethyl alcohol prior to staining.

Following fixation in equal parts ether and 70 per cent ethyl alcohol for a minimum of 15 minutes, the smears were:

1. Hydrated through alcohols (80, 70, and 50% alcohol) to distilled water.
2. Immersed in 1.0 per cent acetic acid for ten seconds.
3. Washed in distilled water for one minute.
4. Stained in 0.01 per cent acridine-orange (Gurr) in phosphate buffer at *p*H 6.0 for one and one-half minutes.
5. Destained in the same phosphate buffer for one and one-half minutes.
6. Differentiated in 1-molar calcium chloride for four minutes.
7. Returned to phosphate buffer for one minute.
8. Mounted in buffer.

The smears were examined in a semi-darkened room with a fluorescence microscope, using wavelengths of 500-800 millimicrons (blue-violet radiation) and orange barrier filters in the oculars. The smears were scanned at a magnification of 125, without the use of a mechanical stage. The mean examination time was less than one minute for smears which contained only a few cells, and about three minutes for highly cellular smears.

Initially, four smears were examined from each specimen, and destained by immersion for a few minutes in 50 per cent ethanol, followed by restaining by the Papanicolaou method. However, considerable sediment was lost during this double staining procedure. Subsequently, only two of the four smears were examined by fluorescence microscopy, and the other two were processed directly by the Papanicolaou technic.

Over 500 urine specimens were examined, but those from other than bladder neoplasms were excluded from the study. This left 478 specimens from men who either did not have a neoplasm of the urinary tract, or had histologically con-

* Present address: St. Joseph's Hospital, Lancaster, Pa.

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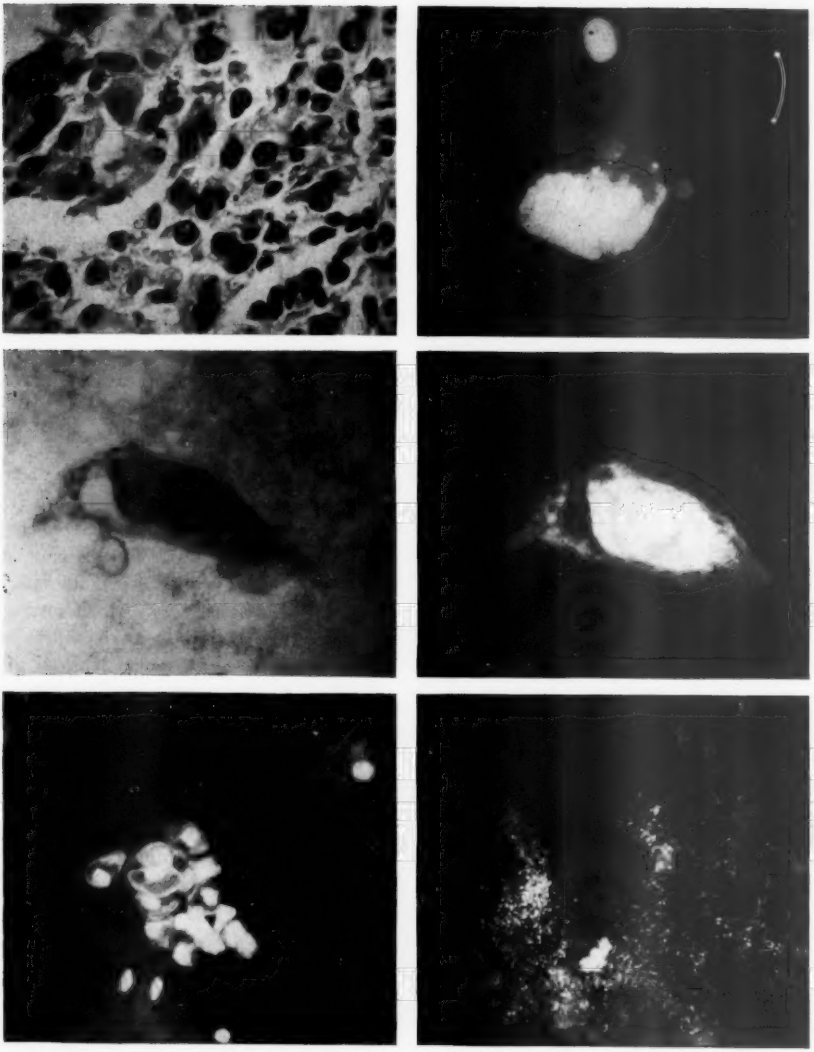

Top Row. FIG. 1. AO-FM. Small cluster of brilliant carcinoma cells in upper-center area. Less bright group below and to left. Both clusters are bright orange, contrasting with the small green leukocytes (x70). FIG. 2. AO-FM. Large cluster of atypical transitional cells from atypical papilloma. Note contrast with dull squamous cells at periphery of photograph (x260). *Middle.* FIG. 3. AO-FM. Brilliant, binucleated, atypical transitional cell. Juxtanuclear vacuole in cytoplasm (x680). FIG. 4. Papanicolaou stain. Same cells as in Fig. 3 (x600). *Bottom.* Fig. 5. AO-FM. Poorly differentiated, degenerating carcinoma cell from invasive neoplasm. The nucleus is brilliant yellow, the cytoplasm is green (x680). FIG. 6. Same case as Fig. 5. H & E stain of histologic section. Poorly differentiated pleomorphic cells (x300).

firmed bladder papilloma or carcinoma. A total of 410 specimens were from patients who had no cystoscopic evidence of bladder neoplasm, 27 from patients who had simple transitional cell papilloma, and 41 from patients with transitional cell or squamous cell carcinoma.

The results of the examinations were recorded as positive, suspicious, or negative.

Results

The sediment from normal urine contained only scattered transitional, cuboidal or squamous cells, and rare leukocytes. Since these cells appeared pale olive-green when examined by acridine-orange fluorescence microscopy (AO-FM), the smears could be screened completely in a few seconds. Red blood cells even when abundant did not alter the appearance since erythrocytes do not fluoresce. Overstaining or excessive alkaline buffering resulted in bright yellow fluorescence of the leukocytes which interfered with the examination, especially when the "pus" cells formed large clusters.

Malignant cells exhibited brilliant fluorescence against the black background (Fig. 1-3, 5). Their cytoplasm and nucleoli were intense orange, the nuclei brilliant yellow or green. More mature *squamous* carcinoma cells or *degenerating* malignant cells (Fig. 1) exhibited less fluorescence, particularly of the cytoplasm which was pale green. Nuclear fluorescence of degenerating cells was more persistent although less intense, and was green rather than yellow. Naked nuclei were common. Vacuolization of the cytoplasm of degenerating carcinoma cells was a frequent feature (Fig. 3). The vacuoles appeared as non-luminescent globules and often displaced the nuclei, producing forms which could not be differentiated from the signet-ring cells of adenocarcinomas.

Three nonmalignant cell types also demonstrated increased metachromatic fluorescence. (1) Some histiocytes exhibited bright fluorescence, although their cytoplasm usually was dull brown with bright orange particles of ingested bacteria. These cells were uncommon in urine specimens and rarely presented diagnostic difficulties because of their characteristic morphologic features. (2) Cuboidal cells, probably from the prostatic urethra or prostatic ducts, were present infrequently, and fluoresced brightly. These cells were also recognized easily because of their characteristic shape. (3) Hyperplastic or regenerating transitional cells caused the most difficulty in interpretation. In general, their appearance was intermediate between that of normal urothelial cells and carcinoma cells. Color differences were of intensity rather than of wavelength. Occasionally, atypical benign cells were brighter than degenerating carcinoma cells or more mature malignant squamous cells. Differentiation usually was possible in these instances by study of morphologic features, but with less certainty than in the Papanicolaou preparations.

In the instances in which the smears contained these equivocal cells, the examination of sequential specimens was helpful. When the atypism was due to inflammation or to the trauma of calculi, the abnormal cells decreased and often disappeared during treatment or after removal of the stones. In the presence of neoplasm, on the other hand, the atypism persisted or increased. In fact, the mere examination of multiple specimens increased the likelihood of finding unequivocal malignant cells.

Benign epithelial cells when coated by bacteria appeared bright orange, but could be recognized by the absence of bright nuclear fluorescence, and the identification of the bacteria when examined at higher magnifications.

In general, the AO-FM findings corresponded with those of the Papanicolaou controls (Fig. 3, 4). Nuclei which stained deeply by the hematoxylin of the Papanicolaou stain exhibited proportionately increased fluorescence. Nucleolar staining by

AO-FM was especially striking, the orange nucleoli and parachromatin granules contrasting sharply with the green or yellow deoxyribonucleic acids of the chromatin. In contrast, in many of the Papanicolaou controls, the nucleoli were so cyanophilic that they could not be differentiated from clumps of chromatin.

The most significant difference between the two technics was the relative importance of the appearance of the cytoplasm, which in the Papanicolaou method is utilized chiefly for determination of cell type. In contrast, the flaming orange fluorescence of the cytoplasm of carcinoma cells was the most striking diagnostic feature of carcinoma by AO-FM. The cyanophilia of cytoplasm in Papanicolaou smears was proportionate to the intensity of orange fluorescence.

The cytologic diagnosis of simple transitional cell papilloma by the Papanicolaou method was difficult unless the tumor included some bizarre cells, since most of its cells could not be differentiated from hyperplastic or even normal urothelial cells. Cells suggesting neoplasm were found in only one-half of the urine specimens from patients with simple or "benign" papillomas. The same problem was encountered in the smears examined by AO-FM. The mature neoplastic cells exhibited a degree of luminescence which was no greater, or even less, than that of the hyperplastic cells found in patients with cystitis or calculi. On the other hand, cells harvested from *atypical* papillomas (Fig. 2) and *papillary carcinoma in situ* (Fig. 5, 6) demonstrated the usual fluorescent criteria of malignancy and were recognized readily.

There was 86 per cent agreement between the results of the two technics in patients without bladder neoplasm. There were four false-positive (1.0%) by AO-FM and two (0.5%) by the Papanicolaou method. Fifty (12%) of the 410 specimens from tumor-free patients contained cells which were interpreted as suspicious by AO-FM, while only 31 (7.5%) of the control Papanicolaou examinations were recorded as suspicious.

In 87 per cent of the specimens from patients with papillomas or carcinomas, the two technics produced identical findings. Carcinoma cells were not found in 25 (6.5%) of the AO-FM examinations and in 17 (4.3%) of the Papanicolaou studies. A review of the smears from the eight specimens which were negative by AO-FM, but positive by the standard method, revealed that the smears in these cases contained very few malignant cells, only degenerated cells, or consisted of large masses of poorly spread sediment which did not permit expression of the fluorescence. On the other hand, some degenerating or distorted cells which were recognized in the Papanicolaou preparations with difficulty maintained brilliant fluorescence.

Discussion

The application of AO-FM to exfoliative cytology is based upon (1) the high protein synthesis of malignant cells, and (2) the metachromatic staining of nucleic acids, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) by acridine-orange, the intensity of the staining being proportional to the quantities of these moities. In general, benign cells in urinary sediment exhibit minimal fluorescence since they are derived from epithelium which normally has relatively low protein metabolism. Malignant cells, reflecting the increased mitotic activity and high protein synthesis of the neoplastic tissue, exhibit brilliant fluorescence which makes their recognition simple.

The normal epithelial lining of the collecting channels of the urinary tract has great capacity for regeneration. Therefore, in the presence of severe inflammation (cystitis) or trauma (calculi) the urothelium proliferates rapidly, and sheds hyperplastic cells into the urine. Since these desquamated cells represent cells with in-

creased metabolic activity, their nucleic acid content is considerably greater than normal, and this is reflected by increased luminescence after AO staining. Although the intensity of fluorescence of these regenerating or hyperplastic cells does not approach that of *typical* carcinoma cells, it may exceed that of degenerating malignant cells or well-differentiated squamous carcinoma cells. Fortunately, in most instances proper identification can be made by morphologic features. In some, this differentiation is established with greater certainty by the Papanicolaou stained preparations, accounting for the lower incidence of suspicious reports with the latter. It was noted that the urine of patients with severe chronic cystitis, especially cystitis glandularis, was most likely to contain these atypical cells. Histopathological sections from these bladders exhibited marked atypical hyperplasia, with bizarre cells as well as increased numbers of division figures. The question of a premalignant propensity of such lesions remains moot.

Summary

In the detection of neoplastic cells in the urine of patients with bladder neoplasms, the results of acridine-orange-fluorescence microscopy technic correlated closely with those of the standard Papanicolaou method.

Despite the rapidity of screening by the fluorescent method, its sensitivity was almost equal to that of the standard method of examination.

Some degenerated carcinoma cells and well-differentiated malignant squamous cells failed to exhibit bright fluorescence but other malignant cells which were difficult to recognize in the Papanicolaou preparations were detected easily by fluorescence microscopy.

Regenerating or hyperplastic urothelial cells from patients with chronic cystitis or urinary calculi were occasionally confused with cells from simple papillomas or well-differentiated transitional cell carcinomas.

Acknowledgment

The author wishes to acknowledge the technical assistance of Miss Renee Soureñne, Mrs. Pearl Hill, and Mr. Darrell McCaslin.

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Morphology of Benign Cells as Observed through the Acridine Orange Fluorescence Technic*

DEWITT T. HUNTER, JR., M.D., NELLO BROWN, M.D.

From the Department of Pathology, Baptist Memorial Hospital, Oklahoma City, Oklahoma

ALTHOUGH it is not within the scope of this study to categorically compare the conventional light microscopic approach with that of the fluorescence approach in cytologic diagnosis, four contrasting differences should be cited (Table 1).

In our hands the technic of staining and clearing requires only seven minutes with less than two minutes being required to scan a preparation.

The fluorescence technic is based on differential analysis of intracellular Ribonucleic Acid (RNA) and Desoxyribonucleic Acid (DNA) content. These nucleic acids can be readily stained with Acridine Orange (AO). RNA, which is increased in malignant cells, fluoresces a brilliant red, while DNA, which is a normal intra-cellular constituent, fluoresces green.[1] Malignant characteristics can be unmasked in cells lacking malignant morphologic characteristics and benign cells having highly atypical appearance can be correctly labeled benign.

Other applications of the fluorescence approach which appear somewhat superior to conventional light microscopy are in the area of acid fast,[5] parasitic, fungus, and virus detection.[3] Preliminary work in hematologic interpretation and as an adjunct in difficult frozen section diagnosis indicates that the fluorescence procedure has broad applications. Evidence to date strongly suggests that the degree of red fluorescence correlates well with the degree of radiosensitivity[2] (Table 2).

One of the two criticisms often leveled against the fluorescence technic is that it is too sensitive and occasionally false positive interpretations will result. Two factors may be responsible for this apparent sensitivity: (A) we may indeed be identifying malignant cells before they can be so identified by other approaches; (B) perhaps some normal cells are sufficiently high in RNA as to approach that of some malignant cells. Concerning the former possibility, it should be noted that until the concept of carcinoma *in situ* was firmly established, the Papanicolaou technic was considered by many to be unreliable because of an unduly high percentage of false positives. It is possible that the fluorescence procedure detects carcinoma while it is still in the basal cell hyperplasia Grade III stage. It will require years of careful study to prove or disprove this concept. As a corollary to the above untoward characteristic of fluorescence analysis is the comforting assurance that false negatives are extremely rare. In an effort to clarify the latter criticism that some normal cells may show deceptive red fluorescence, we have undertaken a fluorescence analysis of all normal tissues in the body and of all normal body discharges. The results of this study are reported below.

The second criticism against the fluorescence technic concerns certain of the staining procedures and the apparatus now in use. At such time as an inexpensive, reliable, versatile light source is available, the

* Presented at the Eighth Annual Meeting of the Inter-Society Cytology Council, 1960, Chicago, Illinois, U.S.A.

TABLE 1. *Brief Comparison of Papanicolaou and AO Methods*

	AO	Papanicolaou
1. Time consumption	Less than 10 mins.	Over 10 mins.
2. Principle of analysis	Histochemical and morphologic	Morphologic
3. Versatility and application	See Table 2	Primarily exfoliative cytology
4. Sensitivity (false positives)	Occasional false positives (Cervical smears)	Rare false positives (Cervical smears)

technical problem will resolve. Certain methods of staining now in use may also contribute nonspecificity.

Materials and Methods

Smears of all human tissue and byproducts were smeared and processed in accordance with the modified von Bertalanffy AO technic.[4] These were scanned and representative fields were photographed. The ultraviolet light source used was the Eclipsoid Mercury Vapor and the camera was a Leica 3c. Ektachrome Type B film was exposed for two minutes. Various microscopes were used and found to be satisfactory. Cell types of different tissues were studied and their characteristics as demonstrated by the AO technic were tabulated. Identical or representative fields were studied by the method of Papanicolaou in the interests of comparing morphology demonstrated by the two methods. Only thin fields were observed since large cell clumps are difficult to analyze by both technics.

Results

In general, most normal mature cells showed green fluorescence while normal immature cells showed greater or lesser amounts of red coloration. In most instances, morphologic detail including distribution of RNA was characteristically benign. These benign characteristics are tabulated in Table 3.

Most rapidly proliferative tissues will show some red fluorescence and occasionally atypical morphologic characteristics will be encountered. A systematic study is now in progress to investigate fully the fluorescence changes seen in metaplastic, inflammatory and other benign conditions.

Table 4 lists all tissues and materials studied. The degree and color of fluorescence is indicated. RBC's, mucoprotein, fat, and non-living materials do not fluoresce.

Summary and Conclusions

The following normal tissue cells show moderate to slight red fluorescence when stained by the AO fluorescence technic: trophoblasts, lymphocytes and precursors, monocytes, myelocytes and precursors, mesothelial cells, hepatic cells, histiocytes, endometrial cells, giant cells, rectal mucosa, granulosa cells, prostatic epithelium, renal epithelium, cartilage and basal cells of mucous membranes. All other normal tissues studied show yellow or green fluorescence. Despite the reddish fluorescence

TABLE 2. *Applications of AO Procedure*

Touch preparation cytologic diagnosis
Intracellular proporphyrin analysis
Intracellular inclusion detection
Exfoliative cytologic diagnosis
Radiosensitivity evaluation
Fluid cytologic diagnosis
Acid fast detection
Parasitic detection
Mycotic detection

TABLE 3. *Comparison of Benign and Malignant Characteristics*

Benign	Malignant
NUCLEI	
Single	Single-multiple
Green	Yellow-green
Homogeneous-chromatin	Clumped chromatin
Small round-oval	Large irregular
No nucleoli	Red nucleoli
Uniform population	Pleomorphic population
CYTOPLASM	
Increased quantity	Decreased quantity
Green colorless	Red orange
Dull coloration	Bright coloration
Round oval outline	Irregular cell outline
Uniformity of size	Pleomorphic size
Intact cells	Degenerated cells
Discrete cells	Clumps of cells
Polar inclusions	Nonpolar inclusions
Homogeneous coloration	Peripheral clumping of RNA

demonstrated by some normal cells, morphologic characteristics are sufficiently definite to enable their differentiation from malignant cells. The applications of the fluorescent technic in fields other than exfoliative cytology are cited briefly.

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TABLE 4. *Staining Properties of Normal Cells*

Red	Yellow	Green
Trophoblasts	Basal cells (All ectodermal surfaces)	Epithelial cells (superficial)
Blast cells	Chronic inflammatory cells	Chondrocytes
Mesenchymal cells	Renal epithelium	Adrenal
Mesothelial cells (reactive)	Cartilage matrix	Gastrointestinal mucosa
Liver cells	Ureteral mucosa	Breast epithelium
Histiocytes	Thyroid	Bladder mucosa
Endometrium	Granulosa cells	Prostate epithelium
Monocytes	Plasma cells	Striate muscle
Rectal mucosa	Skin appendage elements	Heart muscle
Giant cells	Germinal epithelium testes	Smooth muscle
Megakaryocytes	Lymphocytes	Central and peripheral nerve tissue
	Luteal cells	Semen components
		Salivary gland
		Nasal mucosa
		Parathyroid cells
		Lipocytes
		PMN leukocytes
		Gallbladder mucosa
		Blood vessel wall
		Melanophores
		Neval cells
		Amnion cells
Non-fluorescent	**Brilliant red fluorescence**	Pituitary (rabbit)
Red blood cells	Bacteria	Pancreatic cells
Feces	Parasites	Bronchial epithelium
Mucoprotein	Viral inclusions	Ocular tissue
Fat	Fungi	Serous membranes
Vitreous humor		Synovia
Urine contents		Peritoneum
(Non-cellular components)		Pleura

Symposium on Probable or Possible Pre-Malignant Lesions

IV: Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia
(Continued from March-April issue)

Histochemistry of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia

JEAN BERGER
Basel, Switzerland

WITH the aid of histochemical methods, an increased cellular activity, equal to increased growth, can be proven unequivocally. While histological staining methods give information only about the structure of the tissue, additional criteria for the prognostic evaluation are offered by demonstration of certain substances or enzymes.

In the histological section of basal cell hyperplasia we find an increase in the number of nuclei. In this condition, with the help of the methyl-pyronin stain or the toluidine-blue stain we find a marked increase of the RNA and DNA (Fig. 1, 2) — the RNA stains red and the DNA stains blue. By the same method we find an increase in the phosphatases, which are essential for the synthesis and metabolism of the glycogen and nucleo-proteides (Fig. 3).

In another case we show the serial sections from an increased atypical epithelium (Class IV of Hinselmann), which indicates growth of anaplastic proliferating epithelium into the connective tissue or a gland, presented with the various histochemical reactions. The strong growth potential can be demonstrated by the increased cyanophilia with the toluidine-blue stain, as well as by the increased RNA and DNA contents, as revealed by the methyl-green-pyronin staining (Fig. 4-8).

In the detailed pictures (Fig. 7, 8) the increased number of nucleoli and the respective increase of RNA can be recognized.

The acid phosphatase is increased in all layers, not only in the basal layer (Fig. 9). Figures 10 (glycogen stain after Bauer) and 12 (PAS, showing loss of the neutral mucopolysaccharides) demonstrate clearly that the cells in atypical epithelium have lost

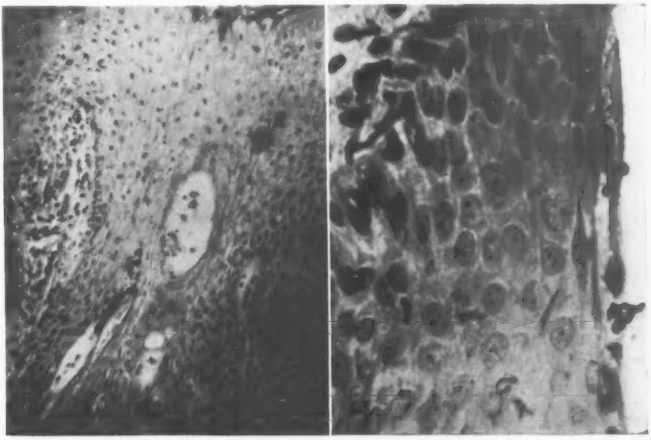

FIG. 1. Basal cell hyperplasia. FIG. 2. Detail of Fig. 1.

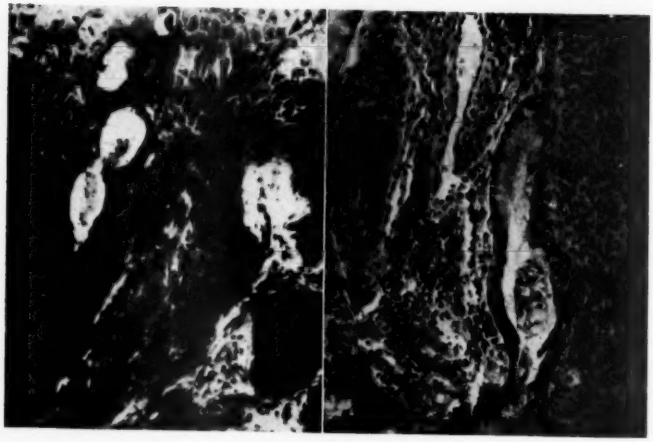

FIG. 3. Basal cell hyperplasia, Gomori technic: acid phosphatases highly increased in growing basal cells and plugs. FIG. 4. Hematoxylin-eosin stained section showing marked atypical epithelium (Class IV of Hinselmann).

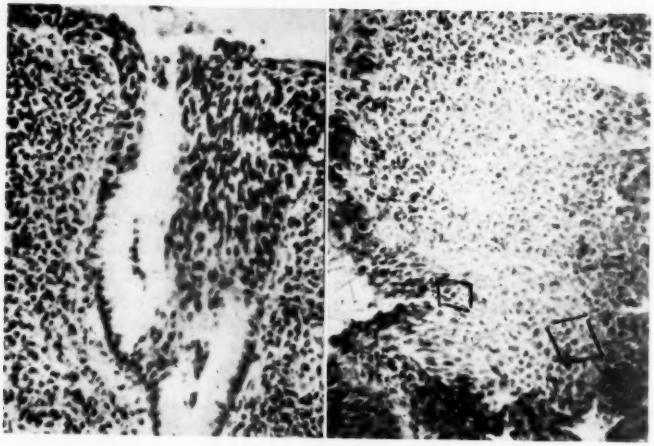

FIG. 5. Marked atypical epithelium, note the strong cyanophilia, indicating increased contents of nucleoproteides. FIG. 6. Marked atypical epithelium, methyl - green - pyronin stain showing increased amount of DNA and RNA.

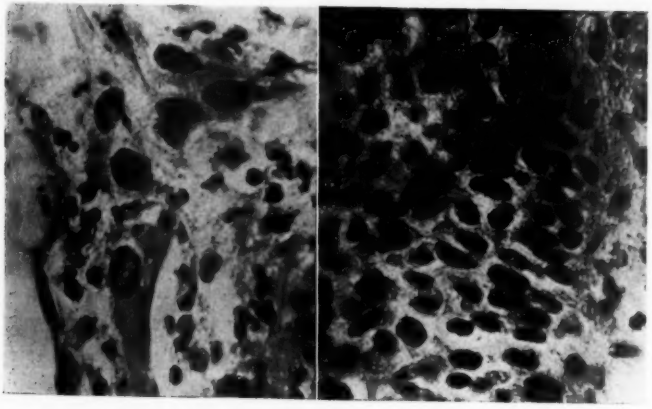

FIG. 7. Detail of Fig. 6.
FIG. 8. Detail of Fig. 6.

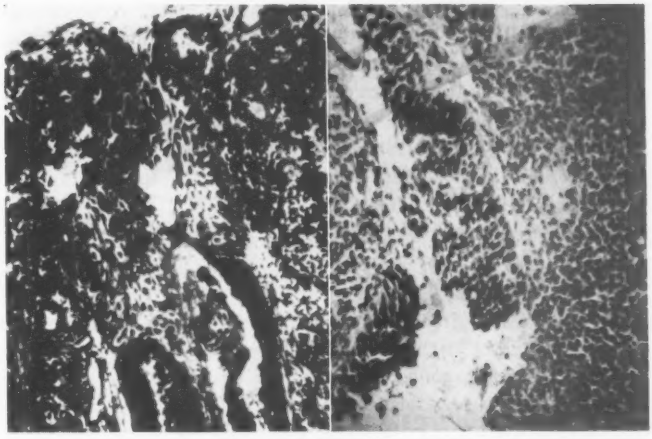

FIG. 9. Marked atypical epithelium, Gomori reaction for acid phosphatase, enzyme increase in all layers. FIG. 10. Marked atypical epithelium, glycogen stain after Bauer. No glycogen present.

the capacity to form glycogen. Only the basement membrane consisting of acid mucoproteides can still be recognized as can be seen in Figure 13 (alcian-blue stain, absence of acid mucopolysaccharides). That we deal here not with an invasive carcinoma, which exhibits the same histochemical staining reactions, can only be proven by the demonstration of a basement membrane with the alcian-blue stain (Fig. 13) or, for instance, by the silver impregnation after Hortega (Fig. 11) which shows the presence of subepithelial silver fibrilles.

Therefore, histochemical staining meth-

FIG. 11. Marked atypical epithelium, Hortega silver impregnation. Demonstration of the subepithelial connective tissue.

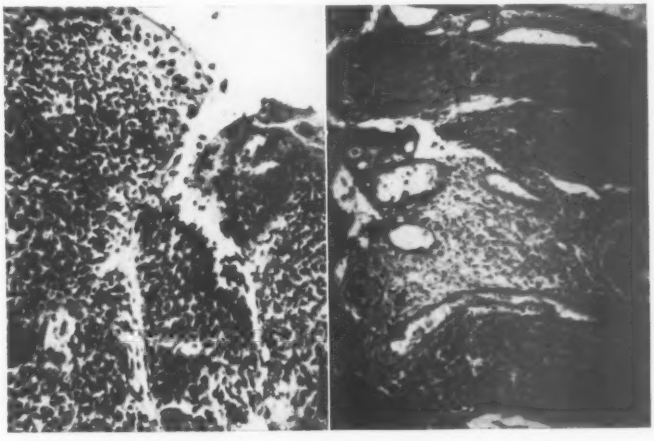

FIG. 12. Marked atypical epithelium, PAS, negative reaction for neutral mucopolysaccharides. FIG. 13. Marked atypical epithelium, acid mucopolysaccharides are absent. The basement membrane, consisting of acid mucoproteides, can be recognized.

ods in basal cell hyperplasia and increased atypical epithelium reveal that there is more than a mere anaplasia present. First we obtain information about the increased growth intensity of the tissue, as revealed by the increase of RNA and DNA and the acid phosphatase, and second we learn about the decreased functional abilities by demonstrating the absence of glycogen and polysaccharides. Prognostic statements can only be made by repeated controls and comparison of the results.

ALVAN G. FORAKER
Jacksonville, Florida

AS NOTED earlier in this Symposium, histochemical studies of the reactions of squamous cells have suggested correlation with growth or maturation, regardless of specific cell type or biologic potential.[1] Cells of reserve cell hyperplasia exhibited marked evidence of dehydrogenase activity,[2] but this probably correlated with their growth state. Phosphoamidase and dehydrogenase[1] were found in various patterns of growing squamous mucosal cells. Glycogen[3] was found in various patterns of maturing squamous cells. In two new studies, dysplasia had more nearly the properties of intraepithelial or invasive squamous cell carcinoma than the properties of metaplasia or normal exocervical mucosa. These included weight of nuclear material in a standard sample of squamous epithelium as determined by interference microscopy and planimetry[4] and characterization of basic and acidic amino acid distribution by the "*p*H signature" technic.[5] Growth versus maturation are the determining factors in histochemical reaction patterns of squamous cells. Until we can find some technic which will differentiate physiologic from pathologic growth, progress in this field will not go beyond a certain point.

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Fluorescence Microscopy of Hyperplasia in Gynecological Cytodiagnosis*

LUDWIG VON BERTALANFFY
Topeka, Kansas, U.S.A.
FELIX D. BERTALANFFY, ALEXANDER M. GOODWIN
Winnipeg, Manitoba, Canada

THE ACRIDINE orange fluorescence method introduces a new cytodiagnostic criterion, namely, changes in ribonucleic acid (RNA) content of the cytoplasm during hyperplasia and carcinogenesis. With increasing concentrations of RNA, the fluorescence of the cytoplasm ranges from brown through

* This work is supported by a research grant from the National Cancer Institute of Canada.

reddish brown and orange to flaming red.

Rapidly proliferating cells are characterized by large amounts of cytoplasmic RNA. Consequently, hyperplastic and malignant cells show orange to red fluorescence of varying intensity. They thus contrast greatly normal cells with greenish (superficial squamous cells) or brownish (basal and parabasal cells) to reddish brown (endocervical cells) fluorescence.

The following remarks are limited to changes of the cytoplasmic fluorescence in hyperplasia.

Hyperplastic basal and parabasal cells, owing to increased RNA content, may show a more vivid reddish fluorescence than their normal brownish cytoplasm. The otherwise normal morphology of these cells, such as their size, shape, normal nuclei and nuclear-cytoplasmic ratio, indicates their non-malignant nature.

"Active" endocervical cells present, for example, in cases of cervical erosion, often show bright red cytoplasmic fluorescence with otherwise normal morphology; normally, endocervical cells have reddish-brown cytoplasm. The increased RNA content and cytoplasmic fluorescence of these cells in erosion is probably related to their more active proliferation.

Increased cytoplasmic fluorescence of basal, parabasal and endocervical cells may also be apparent in Trichomonas and Monilia infections. With the acridine orange fluorescence method, Trichomonas cells and Monilia mycelia and spores appear in brilliant reddish brown and bright red fluorescence respectively; therefore, they are recognized more readily than by any other method. Atypical cells which may occur in such infections usually are readily distinguished from suspicious cells by examining their nuclear morphology, which is clearly demonstrated by the fluorescence method. Moreover, in doubtful cases, the infections should be cleared up before the final diagnosis is made.

Dysplasia cells are likewise sometimes characterized by increased RNA content and red cytoplasmic fluorescence, and show atypical morphology.

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Animal Experiments

EMMERICH VON HAAM
Columbus, Ohio, U.S.A.

VON HAAM AND SCARPELLI,[3] Reagan,[2] Murphy[1] and many others who have produced experimental carcinoma of the cervix in white mice have described stages of severe epithelial dysplasia which nearly always preceded the appearance of invasive carcinoma. In our experiments the stage of dysplasia appeared relatively early, sometimes within four weeks after application of the carcinogen, and exfoliated dysplastic cells made their first appearance during the diestrous stage of the animals. Cellular abnormalities were found in parabasal and intermediate cells and were less distinct in the superficial cells. When the animals went into the high estrous phase of their weekly cycle the dysplastic cells often disappeared from the smear. The appearance of these dysplastic cells was usually preceded by a period of inflammatory reaction which began a few days after administration of the carcinogen. The inflammatory process usually had reached its peak and had begun to subside at the time cellular dysplasia became apparent. The type of dysplasia found was quite similar to the one observed in human cervical smears. Again cytoplasmic changes were more noticeable than nuclear changes and consisted of the appearance of cytoplasmic granules or vacuoles, enlargement of the cells, and changes in the staining quality of the cytoplasm. After some oscillation dysplastic changes finally became definitely established and persisted throughout the cycle. Withdrawal experiments showed that these changes are reversible and may disappear after the administration of the carcinogen has been stopped. However, a certain percentage of the animals did develop tumors in spite of the fact that no malignant cells were observed in the smears before withdrawal of the carcinogen. Nuclear changes of experimental cervical dysplasia consisted primarily in swelling of the nucleus, which often assumed a vesicular appearance, some marginal clumping of the chromatin but without nuclear hyperchromasia or abnormal nucleoli.

From our experiments we felt that cervical dysplasia could be considered a definite forerunner of carcinoma *in situ*. The lesions will disappear in a large percentage of animals after withdrawal of the carcinogenic stimulus. No definite criteria as to its reversibility can be given at the present time, but it is my impression that as long as the changes are confined primarily to the cytoplasm the lesions are more frequently reversible than after dysplastic cell changes appear in the nucleus.

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Discussion

Irena Koprowska, Philadelphia, Pennsylvania, U.S.A.: Results of the following, yet unpublished, experiments indicate that induced cervical dysplasia of the mouse cervix may progress to invasive carcinoma without additional carcinogenic stimulation.

Experiment I: Five groups, each consisting of ten C3H virgin mice, were subjected to bi-weekly intravaginal paintings with 3-4 benzpyrene by a technic described elsewhere.[1] Vaginal smears were examined weekly. Treatments were carried out for 4, 8, 12, 16, and 20 weeks, according to the group. Results based on histologic examinations are tabulated below:

Incidence of cervical carcinoma	Period of carcinogenic treatment	Latent period of carcinogenesis
0/10	4 weeks	Unknown, but 50 weeks
6/10	8 weeks	50 weeks
10/10	12 weeks	29 weeks
10/10	16 weeks	26 weeks
10/10	20 weeks	24 weeks

Experiment II: Twenty mice of the same strain and age were subjected to identical carcinogenic treatments and cellular examinations as in Experiment I, except that animals were sacrificed for tissue studies at the rate of two per week, starting from the third week after the beginning of benzpyrene applications. Some cervical dysplasia, basal cell hyperactivity, and downgrowth of epithelial processes with their branching out were observed between the fifth and the eleventh weeks. More pronounced dysplastic changes and transitions to carcinoma *in situ* were seen between the thirteenth and seventeenth weeks. Microinvasion usually became replaced by definite invasion between the seventeenth and twenty-first weeks.

According to the above experiments, cervical dysplasia was found in tissues of mice sacrificed upon completion of eight weeks of benzpyrene treatment and progressed in six of ten mice to invasive carcinoma within fifty weeks.

In another experiment,[2] the latency period observed during the progression of cervical dysplasia to invasive carcinoma was considerably reduced from 50 to 28 weeks. This was accomplished by means of 24 bi-weekly applications of Tween 60, described as a co-carcinogen,[3] following termination of an eight-week course of benzpyrene treatments.

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Closing Remarks

Emmerich von Haam: I performed similar experiments as Irena Koprowska and obtained similar results. However, examination of the cervices under ultraviolet light showed that some of the 3-4 benzpyrene remained in the tissues for a considerable period after painting had been stopped so that the dates of discontinuance of the carcinogen were quite uncertain and unreliable. For this reason the experiments were repeated with the string method of Murphy which leaves much less "spilled" carcinogen after string withdrawal.

Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia

ANDRÉ-JULES BRET, FERNAND COUPEZ
Paris, France

HYPERPLASIA of the reserve cells is, in our opinion, related to an abnormal metaplastic process; it is the first stage of a state which is still reversible or which may give rise either simply to atypical epithelium or to a dysplasia. We call it atypical epidermization.

Atypical epidermization is visible very early by colposcopy. It looks different according to its location.

1. On the surface of the ectopy:

It becomes visible only after an acetic acid test. In its early stages it looks either like a set of whitish "coulees" which embrace the ectopic elements or like a discrete whitish cap over them. This picture quickly becomes clearer as the stratification builds up. The progression is maximal at the border area between ectopia and normal epithelium, where a sharp border very

soon appears; later it progresses towards the center of the ectopy.

According to the architecture of this modified ectopic epithelium, the atypical epidermization gives rise to pictures of ground mosaic or white sheets. All of these lesions keep their characteristics during the organizing stage:

They are invisible without acetic acid.

They have a sharp border which separates them from the squamous epithelium.

Their visual intensity is greater at the border than in the center, where they progressively disappear around the columnar villi.

They are iodine negative.

2. At the level of the glands:

The epidermization of the glandular crypts by a normal metaplastic process is invisible by colposcopy. The basal cell hyperplasia results in formation of white peri-orificial halos or of whitish drops after acetic acid.

The whitish halo is related to the incomplete, atypical epidermization of the glandular os; it allows the glandular canal to remain visible.

The circular, whitish drop is related to the complete obstruction of the canal.

Basal Cell Hyperplasia

It seems to us that basal layer hyperplasia does not have any specific colposcopic feature. It is normal during pregnancy; it exists in some dysplasias; it is typical of the beginning of atypical epidermization.

Dysplasia

Dysplasias are always visible in colposcopy. They look like white dots, mosaics, grounds or abnormal red areas.

Although no constant correlation exists between the colposcopical picture and the pathological truth, a certain number of remarks are possible:

1. Visual intensity:

This depends on three factors:

a. the importance of the keratosis or the superficial parakeratosis

b. the cellular density

c. the thickness of the abnormal epithelium

2. Acetic acid test:

The application of a 3 per cent acetic acid solution reveals:

a. dysplasias invisible without its use: either young lesions in the process of development, with unpredictable histological patterns, or old lesions, having regular histological patterns with keratosis or parakeratosis.

b. some more discrete dysplasias, more accurately defined by this test: adult lesions, pathologically benign but with either a basal cell hyperplasia with parakeratosis or average keratosis, or a late ripening with discrete nuclear abnormalities.

c. dysplasias directly visible with the naked eye, which are budding and unchanged after the test: these lesions have either a regular histological pattern with a large amount of keratinization or an irregular histological pattern with discrete keratinization.

3. The colposcopic complex:

Analysis of the pictures permits some classification according to their characteristics:

a. Abnormal epidermization on the way to dysplastic development: white spots, mosaics or grounds at the periphery and an orificial ectopy without other colposcopic abnormalities.

b. Dysplastic scar, non-evolutive: white spots, mosaics or grounds with sharp borders, isolated on a normal epithelium or with series of normal epidermization (vascularization, Nabothian cysts, glandular orifices); Lugol's

test sharply marks the limits of the visible abnormality.

c. Irregular dysplasia, evolutive: white spots, mosaics or grounds without sharp borders, together with congestive red areas, easily hemorrhaged with erosion, and with numerous vessels with or without remainder of normal epidermization. Lugol's test is negative over the borders of the anomaly. The anti-infection treatment does not change anything.

By taking into account the intensity of the pictures, the acetic acid test and the colposcopic complex, it is therefore possible to presume the histological type of the lesions.

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Discussion

Ernst-Helmut Krüger, Halle/Salle, Germany: We agree with the statements of Bret and Coupez. We believe that the unsual or atypical transformation zone, which colposcopically represents the most important and most difficult optical picture of an epithelial surface change on the uterine cervix, is hardly describable, and is appreciable only after long experience, since it is so highly variable. The atypical transformation zone is not identifiable by the iodine test. It is unspecific for iodine, *i.e.*, it remains unstained after iodine application. The acetic acid test has been helpful to us in the colposcopy of dysplasias. In contrast to the normal vessels on the cervical surface, the atypical ones do not disappear after painting with acetic acid, but remain quite visible. They also do not respond to hormonal stimuli.

Colpomicroscopy of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia

Wolfgang Walz
Heidenheim/Brenz, Germany

I. Reserve Cell Hyperplasia

What does it mean? In my opinion, it is the origination of a squamous epithelium by the so-called "indirect metaplasia." In this case the reserve cell hyperplasia is the multilayer epithelium formed from reserve cells of the columnar epithelium. Here we differentiate different stages:

A. The reserve cells are lined up like pallisades, in which case the columnar epithelium above is intact. Colpomicroscopically this stage is not recognizable, as these reserve cells are covered by intact columnar epithelium.

B. Multilayer epithelium ("sieve-like form" by Wespi). In this state the columnar epithelium is either lacking in the production of mucus or it is exfoliated. Thus, the indifferent epithelium lies on the surface and is colpomicroscopically recognizable. Regarding the structure, the original form of ectopy is still recognizable. Frequently the cells are arranged vortex-like. The cytoplasm is usually lightly stained, the nuclei are vesicular and usually possess a distinct nucleolus. The shape of the cells is oval, nearly rhomboid and corresponds in size to parabasal cells (Fig. 1). Frequently, intact columnar cells are situated like islands within such an area. The nuclei are sometimes swollen; however, they are never as distinctly stained as carcinoma cells with which they can be mistaken. Polymorphism and polychromasia, which are frequently

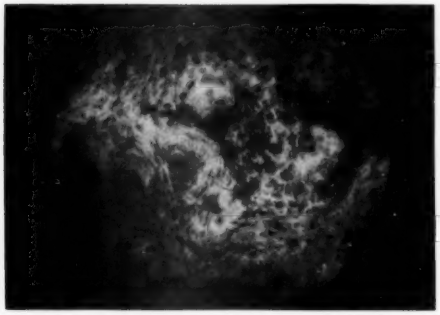

FIG. 1. Surface of an indifferent multilayer epithelium. These and the following pictures stained with toluidine blue.

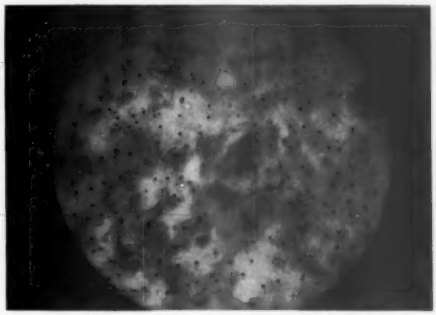

FIG. 3. Basal cell hyperplasia. In the midst of a normal squamous epithelium surface cells; with large, darkly stained, slightly atypical nuclei.

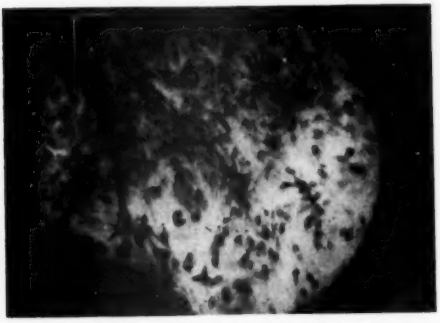

FIG. 2. Indirect metaplasia. Already many parabasal cells. Loose positioning of cells. Left lower border: ectopy.

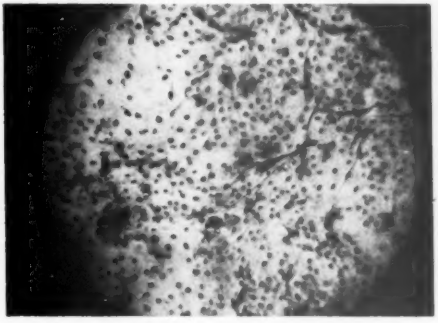

FIG. 4. Dysplasia. Some polymorphism and polychromasia. Chains of spindle cells.

observed, can be explained by the fact that such an epithelium is rapidly developing. Therefore, misinterpretation is possible. However, there are very rarely intact islands of columnar epithelium within a carcinomatous tissue, contrary to observations made on reserve cell hyperplasia.

C. In addition to indifferent epithelium, other cells are to be found which correspond to parabasal cells or lower intermediate cells, depending on the stage of their development toward the squamous epithelium (Fig. 2). Distribution of these cells then becomes looser, the nuclei are stained more intensely and are smaller. This stage resembles the epidermization zone of the normal squamous epithelium (see Topic, Colpomicroscopy of Ectopy, Ectropion and Epidermization).

II. Basal Cell Hyperplasia

I suppose this means basal cell hyperactivity. In spite of being confined to the lower strata of the squamous epithelium this hyperactivity can be diagnosed colpomicroscopically. This is possible because of connective tissue papillae penetrating the squamous epithelium and reaching close to the surface. In this way cells of a striking shape reach the surface (Fig. 3). They possess small, very intensely stained cytoplasm. Their nuclei are considerably enlarged compared to normal basal cells. They are also intensely stained, and somewhat

oval in shape. These cells are grouped in heaps of four to five cells and are located in an otherwise normal superficial layer.

III. Dysplasia

This nomenclature seems to correspond more or less to the "unquiet epithelium" of the Zürich school. Colpomicroscopically there is striking polymorphism and polychromasia, however, not as clearly as in the case of a carcinoma *in situ*. Nevertheless, a differential diagnosis can sometimes be very difficult indeed. Colpomicroscopically, as well as histologically, we can differentiate between two types, one having small cells and the other having large cells with a tendency toward cornification. In the first type (Fig. 4), the nuclei are comparatively small and stain rather darkly. There is a striking abundance of cells on the surface. In our picture there are even spindle cells on the surface. However, there are never atypical cells in spite of the relatively strong polymorphism. Cell boundaries can be traced. The difference between this type and the one with the large cells is mainly the fact that the cell boundaries are clearly recognizable; the nuclei, however, deviate considerably from normal. Usually they are enlarged and possess a number of nucleoli. As a whole, polymorphism and polychromasia are not so strong that they could be mistaken for a carcinoma. However, we believe that histological examination is absolutely necessary in the case of a dysplasia since an apparent dysplasia on the surface could hide a carcinoma *in situ*.

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Clinical Viewpoint on the Management of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia

RAOUL PALMER
Paris, France

HENRIETTE WENNER-MANGEN
Esch-Alzette, Luxembourg

THE LACK of definite knowledge about the evolutionary potential of such lesions leads to the question of their management.

Because our patients often did not return, we abandoned the simple follow up, which is interesting for scientific purposes, and we adopted a more active management.

Since the cervical conoid amputation is an exact and delicate surgical procedure necessitating two weeks of hospitalization, and is not free of complications; it seems to us excessive.

In addition to the hazards of infection and hemorrhage, electrocoagulation is too imprecise and does not reveal the pathology of the destroyed areas.

I. The Excision-Biopsy Performed with the Diathermic Loop

This is of therapeutic and diagnostic value and has many advantages (a) it permits removal, under direct vision, of the exact thickness of tissue desired; (b) it practically does not allow scarring, also less-

ening the risks of hemorrhage and infection; (c) the cicatrization is quick and complete without further stenosis; and (d) it allows the pathological study of the removed tissues including those areas more suspicious than the biopsied area. (This rarely occurs if one uses colposcopy for making the biopsy; it has happened to us once.)

A. The Instrumentation

The choice of the material, loop and electrical source, is determined by the following concerns: (a) to obtain a perfect section without burning the tissues; (b) to obtain a coagulation which is mild but sufficient to obtain immediate hemostasis. Boulite's loop (7 mm. × 10 mm.) is good for this purpose, but it is fragile because of its thinness.

Different apparatus for electrical section can be used if their electrical constants are computed individually. In principle the apparatus with electronic tubes affords a perfect section, with a hemostasis which is often insufficient. The apparatus with sparklers gives an immediate and perfect hemostasis but does not always avoid some tissue coagulation.

B. The Technic

It is very practical to do in an outpatient department. It is not painful and does not require any preparation. As a preliminary measure the cervix is studied by means of colposcopy, smears, and localized (generally multiple), biopsies.

In principle we remove radiated "chips"; the loop enters the cervix 2 or 3 mm. outside the external border of the lesion, cutting down 5 mm. into the tissue and emerges 5 mm. inside the cervical canal. If the lesion is circular and very extended, it is better to perform the procedure twice, separated by a period of two months, in order to avoid any stenosis. A Schiller's test will later verify if the excision has been complete.

The operation must be quick and precise. It may be necessary to electro-coagulate a few bleeding spots.

C. Diagnostic Results

If the apparatus has been regulated well, the histological study as far as it concerns the topography of the lesion (penetration into the connective tissue) is fully satisfactory in the majority of cases.

On the other hand, a very precise cytological study may be disturbed by cellular alterations (especially nuclear), elongations, distortions caused by the action of the electrical current alongside the coagulation zone.

In order to remedy this possible inconvenience, we perform, before the electrical biopsy, localized biopsies with a forceps.

D. Therapeutic Results

The wound (often like a cervical conical excision) cicatrizes in three to six weeks. The cicatrization occurs mainly from the periphery to the center by an overgrowth of the squamous epithelium, without exposing itself to the possible disadvantages of metaplastic regeneration. Simultaneously, a proliferation of the connective tissue occurs, which, after about two months has almost completely filled up the site of the electrical excision.

Almost all the cervices were supple and permeable; their epithelium was absolutely normal without glandular openings or Nabothian cysts; the Schiller test was normal.

In principle, our patients are checked every six months for two years.

The simplicity of the technic and the quality of the cicatrization led us to extend the procedure to adenomatoid hyperplasia and refractory ectopies.

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Discussion

Michael J. Jordan, Genevieve M. Bader and Emerson Day, New York, New York, U.S.A.: From an academic standpoint we are interested in following this group which we categorize as "major atypias" because of the possible potentialities of some of this group progressing to carcinoma *in situ*. In our nine-year prospective study we have yet to see a major atypia progress to an *in situ* lesion histologically, but cytologically there is strong evidence that some of these cases may progress.

In general we would advise minimal therapy in the form of diagnostic curettage and cauterization of the cervix followed by prolonged cytologic follow up at regular intervals with repeat biopsies as indicated.

Closing Remarks

Raoul Palmer and Henriette Wenner-Mangen: We share the viewpoint of the discussants where observation of cervical lesions of the irregular dysplastic type is concerned if, however, the patients are cooperative and intelligent. The follow up on such cases, especially by means of exfoliative cytology should allow us to enlighten the problem of a possible spontaneous regression and to gain some insight into carcinogenesis.

On the scientific level, proceeding by biopsy has brought forward arguments as to the potentialities of various types of intraepithelial lesions reoccurring. All our irregular dysplasias, partly displaying positive or suspicious smears, have from the onset not reoccurred. Two of these cases have had normal pregnancies and deliveries and have had normal cervices and smears in their follow up.

Interrelationship: Reserve-Cell Hyperplasia, Basal-Cell Hyperplasia, Dysplasia and Cervical Carcinoma

JEAN A. DE BRUX, J. DUPRÉ-FROMENT

Paris, France

THE RELATIONSHIPS between these different conditions of the covering epithelium of the ectocervix have been the object of innumerable discussions. Nevertheless, the continuity that relates these lesions may be rather easily followed if we search for their starting point in the epidermoid metaplasia of the columnar epithelium of the ectopies and ectropions. We may in fact consider them as being essentially the result of a disturbance of the epidermization.

The touchstone of these relationships between metaplasia and cancer is the reserve-, or replacement-cell. Depending on the type that these cells will present after their multiplication and superposition, all the architectural possibilities may appear. The two factors which will determine the character of these lesions are:

The differentiation — which governs the general architecture, the stratification and the maturation — which "personalizes" the newly-formed epithelium.

I. Metaplasia

In metaplasia, the reserve-cells, destined to form columnar cells, will be deviated from their original direction: they multiply, become superimposed, and undergo epidermoid differentiation. The germinal cells will give rise to external basal cells which will become intermediate cells with intercellular bridges and a great deal of glycogen; having become superficial, they will then exfoliate, with eosinophilic cytoplasm and pyknotic nuclei, thus realizing the normal maturation of squamous-cell epithelium.

II. Normal Differentiation, Retarded Stratification, with or without Disturbances in Maturation

The lesions falling into this group correspond to the benign or regular dysplasias (simple cellular atypias, Rubrics I and II of Hinselman):

A. Differentiation takes place, but the stratification occurs only very late; hence, the basal layers are hyperplastic but clearly differentiated in their epidermoid character. In the upper one-third of the epithelium the cells mature easily into intermediate and superficial layers.

B. In a certain number of cases, disturbances of the cellular maturation will introduce a new stress which disfigures the epithelium:
The maturation is *precocious,* and there are noted basal cells which, having conserved their prominent nucleus, present an orangeophilic cytoplasm.
Sometimes the maturation is *irregular,* with persistence of immature elements in the upper portion of the epithelium (irregularity and precociousness of maturation are often associated).
Most often the maturation is *excessive,* and there is noted a leukoplakia (keratosic or parakeratosic), also accompanied by a precocious maturation.
We consider the most interesting of these disturbances of maturation, the *dyskaryosis,* associated with an incomplete nuclear maturation: In an epithelium whose basal layers are normal or barely thickened but whose nuclei are hypertrophic and obese, there is noted, in the upper one-half or two-thirds, the presence of large cellular elements whose cytoplasm is poorly limited, usually eosinophilic, but whose nucleus has not followed the maturation: it is voluminous, blown up, with pseudo-"buds" and ridges of retraction, as if pleated. The nuclear membrane is thick and irregular. The nucleolus is bulky, but the rest of the nucleus appears empty. Hence there is a real discrepancy between the cytoplasmic and the nuclear maturation. It should be noted, however, that at the outset the nuclei are hypertrophic and hyperchromatic.

Sometimes the dyskaryosis is associated with a dysplasia. Here there is noted an abrupt nuclear retraction with angular contours and dense chromatin.

III. Varying and Uncertain Differentiation, Retarded Stratification, Very Disturbed Maturation

These three elements dominate the aspect known as irregular dysplasia (aggravated epithelial atypias of Hinselman—Rubric III and IV).

The cells of the lower strata most often have a hyperplastic aspect, and the differentiation is *varying* or *uncertain.* Nevertheless, the structure of cells of basal type is recognizable or at least hinted at. Most frequently these cells are elongated upwards, being turned perpendicular to the basal membrane, but their cellular limits are rather distinct, and the packing is not very marked. The stratification is sometimes retarded and takes place only in the very upper portion of the epithelium.

In these cases, the character of the lesion is determined by the quality of the maturation, which may be:

1. Precocious, with cytoplasmic cornification and abrupt nuclear retraction starting at the first layers.
2. Excessive, with a thick leukoplakia. As in the preceding form, excessive and precocious maturation are often associated.
3. Deviated, or erratic, occurring anywhere at all in the epithelium; in this case it is often also
4. Disharmonious, with elements having mature eosinophilic cytoplasm but absolutely intact nucleus, and vice versa.

TABLE I. *Replacement Cells*

PROLIFERATION

Differentiation	Differentiation	Undetermined differentiation	Differentiation inconstant or absent	Lack of differentiation
↓	↓	↓	↓	↓
Normal stratification	More or less slow (tardy) stratification	Tardy stratification	Stratification absent	Piling of cells
↓	↓	↓	↓	↓
Normal maturation	Disturbed maturation: —Precocious —Incomplete (dyscaryosis) —Excessive (leukoplakia) —Irregular	Disturbed maturation: —Precocious —Excessive —Deviated —Unharmonic	Maturation absent or only slight	Complete immaturity
↓			↓	↓
			Hyperplastic cells few mitosis, few or no anomalies	Nuclear hyperplasia few mitosis, few or no anomalies, inversion of nuclearplasmic ratio
↓		↓		↓
Normal metaplastic squamous epithelium	Regular dysplasias	Irregular dysplasias	Active metaplasia undifferentiated and immature (basal cell hyperplasia)	Carcinoma-*in situ*

TABLE 1A. *Epidermoid Metaplasia*

Normal columnar cell epithelium	Starting malpighian metaplasia	Normal malpighian metaplasia With persistency of columnar metamorphosed cell

TABLE 1B. *Epidermoid Metaplasia*

Undifferentiated and immature metaplasia	Intraepithelial carcinoma

The lesions thus "marked" by the quality of the maturation of their elements raise the problem of their malignant potential.

IV. Absence of Differentiation, Absence or Stratification, Absence or Practically Absent Maturation

In this form, the entire epithelium is covered over by piles of cellular elements placed side by side. Differentiation is hardly perceptible, but may be guessed by examining carefully the cells of the middle zone of the epithelium. The cytoplasmic limit is sometimes rather clearly visible, and on immersion one may discern a sketching-in of intercellular bridges. But, at all the levels, the nuclei remain voluminous, with the nuclear membrane regular and thick; the nucleolus is prominent, whereas the rest of the nucleus is clear. Although the nuclear-cytoplasmic ratio becomes inversed and a few mitotic figures are encountered, nevertheless, there exists no nuclear anomaly suggestive of malignancy.

TABLE 2A. *Active Differentiated Metaplasia and Dysplasia*

Simple basal cells hyperplasia	Dyskaryosis	Regular dysplasia
	Normal differentiation—delayed maturation of "obese"	Normal differentiation and irregular maturation—delayed restriction of hyperplastic nuclei

This is the typical aspect known as hyperplasia of the reserve-cells, which we may rather designate as *active "immature" undifferentiated metaplasia.* Its histological and cytological aspects are extremely deceiving to an inexperienced eye, and have led to countless unnecessary cervical and uterine ablations.

V. Undifferentiation, Immaturity, Anomalies

When the cellular elements constituting the active undifferentiated metaplasia are extremely numerous, they become so densely packed together that the cellular limits are invisible. The cells remain thus densely packed as far as the extreme upper portion of the epithelium, where occasionally there appears a single layer—intermittent—of elongated elements tending toward maturation.

On higher magnification there may be seen, at all levels of the epithelium, rather numerous mitoses—few regular, others abnormal.

Undifferentiation, immaturity, nuclear density, and presence of few anomalies: In the presence of these criteria, one is justified in affirming a carcinoma *in situ.*

Continuity of Lesions

A systematic study of cervices removed for intraepithelial or invasive carcinomas usually shows a continuity between the various lesions just described. There sometimes exists a succession of lesions of increasing severity, at other times they are mingled so haphazardly that next to a lesion of extreme gravity there may exist a simple dysplastic lesion. This aspect points out the starting-point of these lesions: *i.e.,* the multiplication and epidermoid differentiation of the reserve-cells.

A persistence of the undifferentiation and immaturity of the elements sometimes resulting in an over-all picture in which it is difficult to distinguish the carcinoma *in situ* from the hyperplasia of the reserve-cells (immature undifferentiated metaplasia). These, moreover are often distinguishable only by barely-perceptible nuances, which explains the discrepancy often existing between the pessimistic histological report and that of the experienced cytologist (Class II or III). *But this apparent discrepancy gives the key to the diagnosis:* the decisive answer is that furnished by the cytological report, for the intimate details of the chromatin structure are more clearly discernible on the isolated cells of a smear. And the benignity of the lesion is borne out by its subsequent clinical evolution.

We are still in ignorance as to why, on the same cervix, there may exist lesions so disparate. It is, moreover, notable that very often the excised lesions recur, with the same structure and the same anomalies, and at the same location.

Histogenesis of These Lesions

In the Topic on Epidermization we have set forth the facts which cause the undifferentiated elements known as reserve-cells to multiply and undergo an epidermoid differentiation.

TABLE 2B. *Active Differentiated Metaplasia and Dysplasia*

Irregular dysplasia	Irregular dysplasia
By totally precocious and excessive maturation	With deviated maturation and nucleoplasmatic dysharmony

But more important than this would seem to be the question of the cellular undifferentiation and immaturity. The work previously published in this journal on the cervical lesions during pregnancy throws some light on this difficult problem.

Pregnancy determines an activation of the cellular elements of certain tissues embryologically sensitive to the ovarian hormones (endometrium myometrium, connective tissue). We know, moreover, that interruption of pregnancy does not stop this activation, so long as the chorionic villi conserve their hormonal action. Arias-Stella, and later several other authors, have admirably shown the presence, in these circumstances, of endometrial and endocervical cellular atypias sometimes mistaken for cancerous cells.

The hormonal disequilibrium of certain pregnancies may well be the cause of the severe disturbances of the epidermization of ectropions sometimes encountered. In these, the aspect resembles that of the carcinoma *in situ* to such an extent that for some time it was believed that the state of pregnancy itself was a "cancerizing" factor.

It is precisely at this point that experience in cytology comes to one's aid: In these cases, the exfoliated cells present few or no nuclear anomalies, the nuclear membrane is regular, and the chromatin, although abundant, is well distributed; the cells thus resemble more closely very exuberant and very chromatic hyperplastic cells, than carcinomatous cells. This then explains the dramatic regressions observed once the pregnancy is terminated or once its hormonal equilibrium is re-established. Successive biopsies show, in fact, a progressive return to differentiation and maturation, and then the formation of dysplasias, at first irregular, then becoming regular is observed.

These facts enable us: first, to narrow down the riddle of cellular malignancy (without, however, permitting us to solve it); and second, to question the autonomy of the lesion designated as "carcinoma *in situ*" or "intra-epithelial carcinoma."

CARLO SIRTORI

Milano, Italy

THESE PROCESSES are probably chronologically consequent. The first is characterized by the appearance of cells which are similar to those of the basal layers of the ectocervical epithelium. This process is peculiar to the endocervical epithelium. The second shows a numerical increase of the basal cells, sometimes disposed in three to four layers, while most superficial elements preserve their normal feature.

The dysplasia is characterized by a numerical increase of the basal cells which are

irregularly distributed and which sometimes present pictures of self-invasion. When these cells cover the whole thickness of the epithelium and take the place of the normal ones, carcinoma is in question: it is called *in situ* if the basal membrane is undamaged, invasive if it is broken through.

Discussion

Ronald R. Greene, Chicago, Illinois, U.S.A.: We have been following a group of 321 patients with basal cell hyperplasia or dysplastic lesions involving the full thickness of the epithelium, but not sufficiently abnormal to justify the diagnosis of carcinoma *in situ*. Attempts are made to obtain four quadrant biopsies at yearly intervals with the former diagnosis and at six month intervals with the latter. The number of total biopsies on individual patients has varied from two to 15 with 175 of them having been biopsied three or more times. Thirty-one have been followed three or more years (the longest is nine years), 54 for one to three years and the remaining for less than 12 months.

The findings in the last of the repeat biopsies have been negative in 42.7 per cent and a basal cell hyperplasia or a dysplastic lesion in 43 per cent. Fourteen per cent of the lesions have apparently "progressed" to carcinoma *in situ*. Invasive carcinoma has been found in one patient. True progression probably occurred in 11 of the 45 cases with pre-invasive carcinoma *in situ*, since two or more years intervened between the first biopsy and the discovery of the carcinoma.

It seems that these lesions may regress or at least disappear, persist as such and, in a small per cent to date, progress to pre-invasive carcinoma *in situ*. The latter phenomenon has been more common with the full thickness dysplastic lesions (19.2 per cent) than with basal cell hyperplasia (8.4 per cent).

Ernst-Helmut Krüger, Halle a. d. Saale, Germany: We fully agree with the statements of de Brux and Dupré-Froment, especially regarding pregnancy. Each degree of atypia possesses the potentiality of evolution towards carcinoma. Among 12 cases of basal cell hyperplasia, histologically confirmed by ring biopsy, we have seen only one evolve into a so-called "microcarcinoma."

Thomas A. Slate, San Diego, California, U.S.A.: One must compliment de Brux and Dupré-Froment in their most scholarly presentation of such a challenging subject.

We also have been very interested in the potentialities of reserve cells for the past four or five years. We believe that the reserve cells of the endocervix have the same potentialities as the basal cells of the ectocervix, as both are capable of developing into various degrees of atypias or carcinoma *in situ*, depending upon the degree of abnormal differentiation and proliferation. Along the same line of reasoning, we believe that atypical reserve cell hyperplasia (Grade II or Grade III) has the same potentialities as Grade II or Grade III basal cell hyperplasia for the following reasons:

1. Both histologic entities are common in the younger age group—usually one to three years below the average age of carcinoma *in situ*.

2. Atypical reserve cell hyperplasia is frequently associated with and blends into areas of so-called small-cell carcinoma *in situ* (reserve cell type). In fact, we have one case in which serial sections reveal one field of carcinoma *in situ*, apparently developing in a previous field of reserve cell hyperplasia. The slide was reviewed by Hertig who concurred with the diagnosis of focal carcinoma *in situ* probably originating from the reserve cells. Similarly, many cases of carcinoma *in situ* have areas of basal cell hyperplasia adjacent to them or blending into the areas of malignancy.

We agree with de Brux and Dupré-Froment that there is a continuity of lesions or atypias of varying severity in that one commonly finds various degrees of basal cell hyperplasia, or dysplasia, and reserve cell hyperplasia in the same cervix.

Rudolf Ulm, Vienna, Austria: I agree with the statements of the main authors and believe that the epithelial changes in question represent an evolutionary process which deviates from the normal and which may result in a carcinomatous change of the epithelium. It should be emphasized, however, that this evolutionary process, which is partly degeneration and partly growth activity, may be interrupted at any stage of development and that the state thus achieved may persist. Of practical importance is the fact that all of these abnormalities, including dysplasia, are reversible. Only carcinoma *in situ* represents an irreversible epithelial change and therefore, because of the resulting therapeutic consequences, has to be strictly separated from the still benign reversible epithelial changes.

Closing Remarks

Jean A. de Brux: Like the discussants I am convinced that there exists a distinct boundary-line between the dysplastic lesions and the true carcinoma *in situ*, which is irreversible and will inevitably invade. And it is the histological and cytological criteria of these characters which we are trying to determine.

Carlo Sirtori: Greene's results agree with the current opinions: the hyperplastic and dysplastic lesions can precede the carcinoma, but not always and not necessarily do they change into cancer. It has been shown by experimental research that the dysplastic lesions, provoked by the application of carcinogenic substances, regress or do not evolve if the applications of the carcinogenics were discontinued, and only a small percentage will develop into a malignant tumor (Shubick).

Symposium on Probable or Possible Malignant Cervical Lesions: Carcinoma in Situ

I. Histology of Carcinoma *in Situ*

Histomorphology of Carcinoma *In Situ*

FRIEDRICH BAJARDI

Graz, Austria

PARALLEL to the histological diagnosis of an invasive carcinoma is the general view that this disease, should it remain without treatment, will ultimately lead to the death of the patient, due to its boundless destructive growth and with the appearance of metastases. The diagnosis thus corresponds with the forecast which, as experience shows, has usually proven correct, insofar as the diagnosis of carcinoma is correct.

Our own investigations also show that this view of a regular clinical course of the illness in untreated cases holds true also with regard to a diagnosis of carcinoma *in situ*. On the one hand, a carcinoma *in situ* may develop after differing periods of time into an invasive cancer, while on the other hand it may remain stationary for decades as a mere surface disease. For the time being at least, we prefer to rule out of our considerations any spontaneous regression towards a benign epithelium, as far as real carcinoma *in situ* is concerned.

This combination of diagnosis and prognosis may be taken as a working hypothesis, the accuracy of which can be proven only after an investigation of a sufficiently large number of untreated patients. An immediate advantage of this assumption can be drawn from the fact that the investigator is obliged to give the diagnosis carcinoma *in situ* only after a very rigorous examination of facts. It is important that the wide field of suspicious premalignant lesions, the spontaneous regression of which is taken into consideration and in a way justifies a waiting watchfulness without any active therapy, must be distinguished as far as possible from carcinoma *in situ*.

The classical histological symptoms of carcinoma *in situ* were reported in the literature.[12, 13, 16] These criteria were also confirmed by later investigators.[1, 5, 6, 7, 8, 9, 10, 11, 15, 17, 19, 20, 21] E. Siegler,[18] on the other hand, very vividly describes to what extent opinions of pathologists still differ in diagnosing carcinoma *in situ* in practice by reason of frequently including premalignant lesions in a group with carcinoma *in situ*.

The process of the author's histological method of diagnosis is shown by means of microphotographs. The changes of the surface epithelium, as seen in Figure 1, can be considered as a standard model of a carcinoma *in situ*. The most striking fact is primarily the greatly increased number of cells. There is no stratification whatever in the epithelium. The cell borders are blurred so that only nuclei areas can be distinguished. The nuclei are different in size, shape and staining intensity. Sometimes granulations of the nuclei can be observed. There is, too, a considerable increase in the number of mitoses, which are not only to be found in the basal, but also in the middle, and occasionally also in the upper third of the epithelium.

Such standard examples of an undifferentiated carcinoma *in situ* are relatively rare; it is the variations that seem to predominate. Cytomorphologically striking variations in the material examined at our clinic have already been discussed elsewhere,[2] in

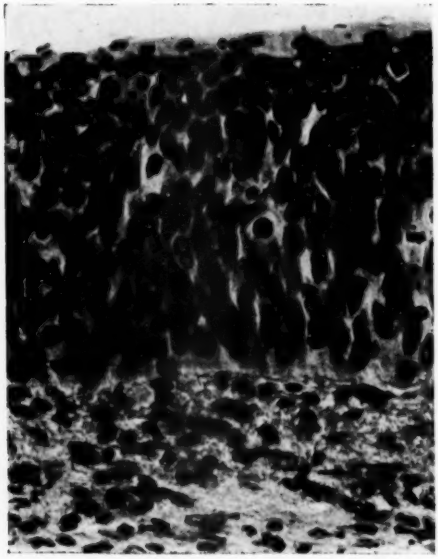

FIG. 1. Typical example of an undifferentiated carcinoma *in situ*.

the course of which we were able to distinguish a small-cellular, a basal-cellular, a spindle-cellular and an especially polymorphocellular carcinoma *in situ*. (The histoogical characteristics are to be found in the nomenclature.)

Examples of a differentiating growth of surface carcinomas are demonstrated in Figures 2 and 3. A further case is described in "Histomorphology of Leukoplakia" in this Symposium. The author believes that the evaluation of such changes causes difficulties and that it depends more upon the personal attitude of the investigator than the diagnosis of the undifferentiated carcinoma *in situ*. In the author's own histological work on the subject, the trend generally is to classify similar changes as "atypical epithelium" and not as carcinoma *in situ*. The reason for this is, on the one hand, that the differentiation of the epithelium stands to some degree in contrast

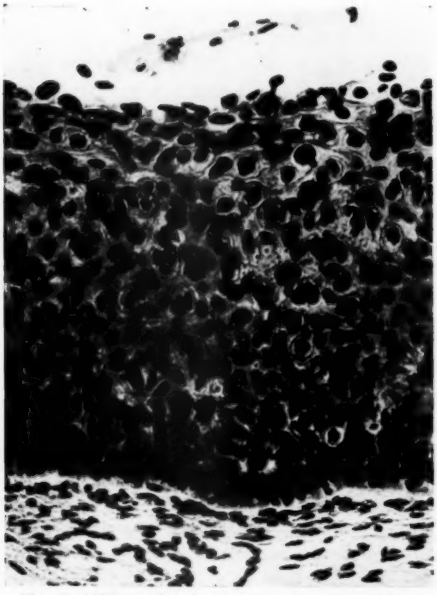

FIG. 2. Differentiated carcinoma *in situ* with numerous mitoses also in the upper layers of the epithelium.

FIG. 3. Differentiated carcinoma *in situ* with especially pronounced granulations of nuclei.

to the conception of carcinoma, since differentiation in general is combined with a reduction in the potency of growth.[13] On the other hand, the findings of early cancer invasion indicate that also differentiated, advanced invasive tumors can have their origin in undifferentiated surface carcinomas. As demonstrated by Fennel,[4] and as we ourselves[3] confirmed in the material examined, a very pronounced differentiation in early invasive cancer buds was often observed, whereas the carcinomatous epithelium of the surface was undifferentiated. Thus there is no real necessity for the assumption that differentiated surface carcinomas are the origin of the frequent differentiated invasive carcinomas. The author, therefore, believes that a certain reserve in giving a corresponding diagnosis is to be employed and that it should be confined to a few very striking changes of the surface epithelium.

In the differentiated surface carcinomas a more or less distinct stratification of the epithelium can generally be observed. The changes shown in Figure 2 caused a diagnosis of carcinoma *in situ* to be given owing to the large number of mitoses, which can be found even in the layers near the surface. In the epithelium shown in Figure 3 the especially pronounced granulations of the nuclei were mainly responsible for the diagnosis.

Mention should be made briefly here regarding the importance of the mitoses in the diagnosis of carcinoma *in situ*. Should they be present in large numbers and should there be also anomalies of mitoses, such facts doubtless present a considerable help in the diagnosis. On the other hand, however, as Meyer[11] already emphasized, an increase of mitoses cannot be taken as basis for the diagnosis. They may even be missing at times.

In conclusion, it should be pointed out that difficulties even in the evaluation of suspicious changes in the epithelium can very often be avoided, if the investigator goes to the trouble of examining step serial sections of a conization. Very often the examination of a single, and even of several sections, does not permit a final decision; only by investigating a sequence of further sections can the diagnosis of a carcinoma *in situ* be given with certainty. Moreover, only by this method is it possible to gain a sufficient knowledge of the extent of a carcinoma *in situ*, located at the ectocervix, the endocervix, or in the cervical glands. Likewise, it is often possible only by this procedure to determine the early invasion of a carcinoma which may be of decisive importance for the choice of therapy to be employed.

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JEAN A. DE BRUX AND JACQUELINE DUPRÉ-FROMENT
Paris, France

IT IS important to place the histological definition of carcinoma *in situ* within very narrow limits. We wish to emphasize its relationship with the active, undifferentiated metaplasia; these two lesions resemble each other so closely in numerous transitional forms that it may be imagined that the carcinoma *in situ* is in some cases the hyperactive form of the undifferentiated metaplasia.

Topographical Morphology

In speaking of carcinoma *in situ* we must have very clearly in mind the notion of a lesion essentially immature, except in the very last cellular layers, which may undergo an imperfect maturation.

Even under low magnification, the lesion is striking for its homogeneity, monomorphism and accentuated cyanophilia. Indeed, it seems to be composed solely of nuclei packed together, all of them oblong, nearly all regular, and all turned perpendicularly to the surface, in nearly perfect order. There is no longer a distinct generative layer, but the intact basal membrane is perfectly outlined, if not slightly accentuated and sometimes even hyalinized. The basal membrane constitutes the lower limit of the lesion, which occupies the entire covering epithelium, whence the term "intraepithelial" (Fig. 1, 2).

Meanwhile, in the subjacent stroma, the glands undergo an identical process, without, however, being suspicious of an invasive carcinoma. For the filling-in of the entire glandular cavity, while finally forming a deeper "digitation," is nevertheless still limited by an intact basal membrane.

Cellular Study

On higher magnification there may be distinguished, around the nuclei, a slender ring of cytoplasm with poorly-defined limits and complete absence of intercellular bridges and the cells are contiguous.

The nuclei, oblong or simply oval, are voluminous, with regular or barely notched contours, and a clear nuclear membrane. The chromatin is divided into tight clumps, leaving apparently no place for a nucleolus. Normal and abnormal mitoses, with abnormal positioning of the chromosomes on the mitotic bundle, are present at all the levels, more especially in the deeper part (Fig. 3). At the surface, an abrupt cellular maturation nearly always occurs, leading to the formation of two or three layers of cells whose stretched cytoplasm is ranged parallel to the surface of the epithelium. Their stretching gives rise to the fiber-cells. The tinctorial affinity, being variable, may be cyanophilic, less often eosinophilic, sometimes polychroma-

FIG. 1. Histological section of a carcinoma *in situ*.

FIG. 2. Histological section of a carcinoma *in situ*.

tophilic. The nuclei, very irregular because they are imperfectly retracted, have angular or festooned contours. The structure of their chromatin becomes invisible, being replaced by an intensely black substance. Around this nucleus, prematurely retracted, a pale halo forms in the cytoplasm.

Briefly, the carcinoma *in situ* is an immature lesion, having no great cellular anomalies, and being composed of nuclei whose packing and density, as well as their ascent in tight columns, is characteristic. Hence, its principal diagnostic character is the presence of cellular immaturity in nearly the entire epithelium.

The irregular dysplasia, which sometimes raises diagnostic problems, is distinguished by the character of precocious maturation in the upper one-half or two-thirds of the epithelium, and by epidermoid differentiation characterized by intercellular spaces and sketching-in of intercellular bridges.

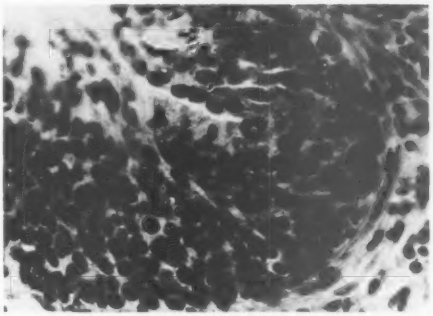


FIG. 3. Histological section of a carcinoma *in situ* with increased number of nuclei and mitoses, in the bottom of a gland.

As to the distinction between active undifferentiated metaplasia (atypical hyperplasia of the reserve cells) and the carcinoma *in situ*, the problem—theoretical and practical—still remains: that of the borderline lesions between benign and malignant changes.

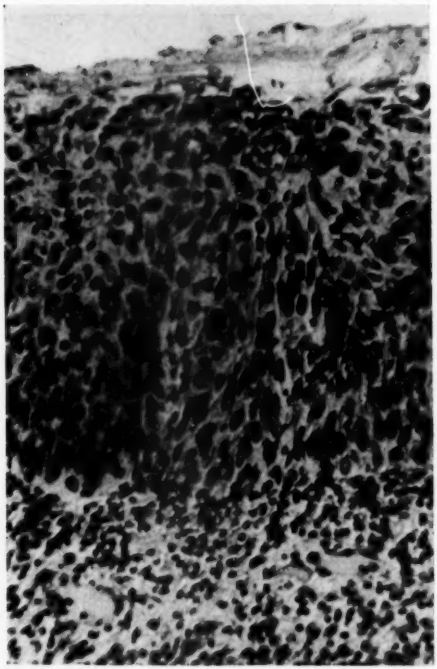
EDWARD E. SIEGLER
Garfield Heights, Ohio, U.S.A.

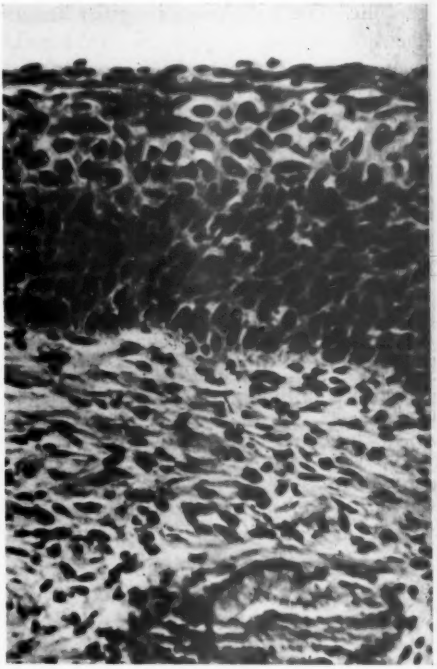
THERE is little doubt that the histologic diagnosis of cervical carcinoma *in situ* is frequently a difficult diagnosis to make with complete certainty. One approach which can be used to help elucidate the various histological patterns containing sufficient criteria for the diagnosis of carcinoma *in situ* is that of comparative opinions of a group of pathologists. If one assumes that multiple opinions are more significant than a single opinion, then one could presumably identify certain histological patterns in which agreement among a significant number of histopathologists would be excellent. One would then have a known baseline to use as control. Included in this group would be some of both the definite positive and definite negative patterns. On those patterns where reasonable agreement is not found, at least one would know that others have similar problems and that these patterns should remain open for future study.

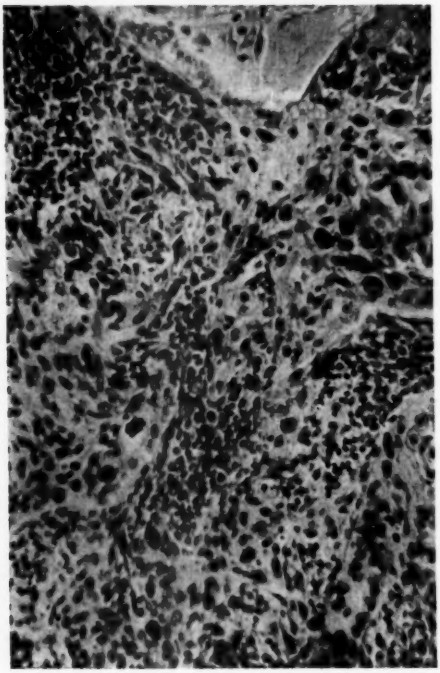
Based on the above plan, 25 cervical slides were examined by 22 pathologists, using the following recommended code:

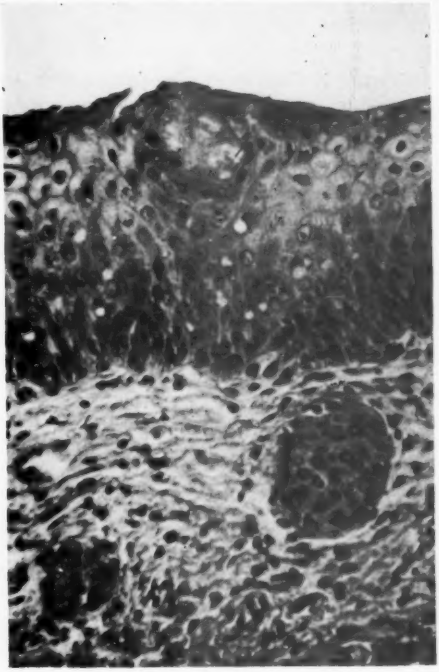
1. Chronic cervicitis } Negative
2. Benign atypia }
3. Borderline (Diagnosis uncertain)
4. Carcinoma *in situ*
5. Carcinoma *in situ* with probable or definite early invasion
6. Invasive squamous cell carcinoma

Due to lack of space, the comparative tabulations cannot be given here. Two conclusions are worth mentioning. Although this collection was practically consecutive, unselected case material, diversion of opin-









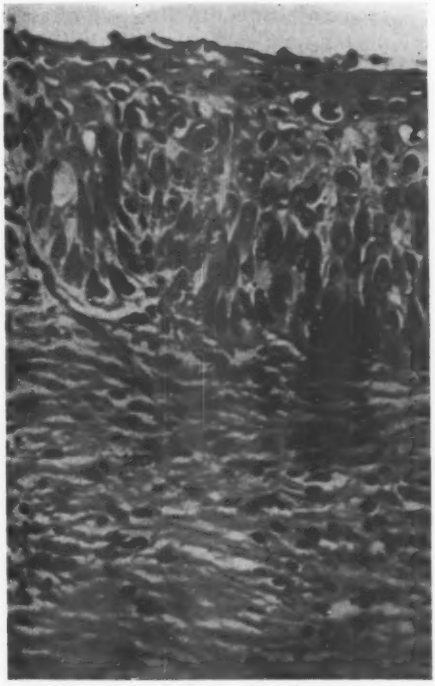


FIG. 5. Nine pathologists placed this slide in the "Diagnosis uncertain" group. Eight preferred the negative category, while five pathologists out of 22 believed this lesion to represent a carcinoma *in situ*. This pattern, therefore, is a controversial one.

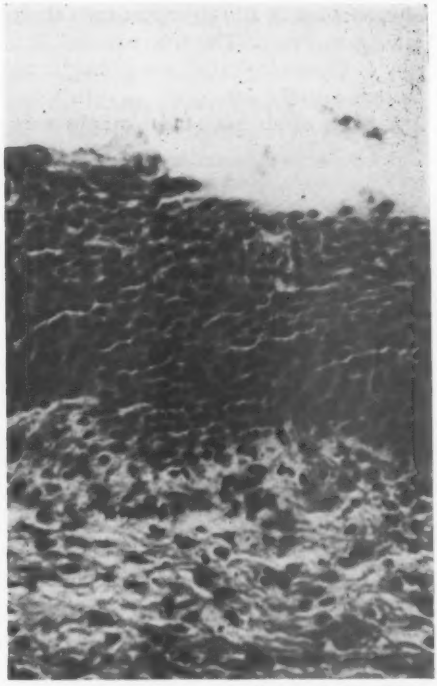


FIG. 6. Half of the group diagnosed this slide as *in situ* carcinoma, six pathologists as borderline and five as negative. The final consensus diagnosis is also uncertain.

ions was quite marked. These cases were taken from a group of three thousand clinically negative cervices, with abnormal cervical smears whose tissues were not diagnosed by our laboratory as chronic cervicitis. The range of totals on the 25 slides were: Total positives, 5-19; carcinoma *in situ*, 2-18; early invasive, 0-7; invasive squamous cell carcinoma, 0-5; borderline, 0-9; and negative, 1-12. The average participant placed 5 out of the 25 slides (20%) in the group labeled, "borderline, diagnosis uncertain," which is highly significant when one remembers that these were almost consecutive unselected cases.

I believe it is very important for some organization interested in attempting to bring uniformity to the histological criteria

FIG. 1. Nineteen out of 22 pathologists diagnosed this slide as carcinoma *in situ*. Two believed it to be *in situ* with early invasion, and one labeled it borderline. This is an example of excellent agreement.

FIG. 2. Eighteen out of 22 pathologists diagnosed this slide as carcinoma *in situ*. One believed it to be *in situ* with early invasion, but three observers interpreted this histological pattern as negative. These latter three might want to reconsider.

FIG. 3. Nineteen pathologists interpreted this as invasive squamous cell carcinoma while three called it carcinoma *in situ* with probable or definite early invasion. This is excellent agreement, but how is questionable early invasion treated at your institution?

FIG. 4. Nineteen out of 22 pathologists interpreted this slide as negative for tumor, two used the borderline category and one interpreted this as carcinoma *in situ* with invasion. This is good agreement except for the last mentioned participant who probably ought to reconsider his opinion.

of carcinoma *in situ* to repeat such studies as suggested here. The International Academy of Gynecological Cytology might well be that organization.

A series of six photomicrographs exemplify some of the results. Except for Case 3, all photos were taken at slightly greater than ordinary high dry (x430) magnification. Case 3 is closer to a low power magnification (x100).

CARLO SIRTORI

Milano, Italy

CARCINOMA *in situ* is spoken of when the epithelium is formed by hyperchromatic cells with scanty cytoplasm and without tonofibrils, and when the basal membrane is not yet broken through and damaged by atypical cells.

CLAUD W. TAYLOR

Birmingham, England, U.K.

PATHOLOGICAL diagnosis depends on two factors—recognition of tissue changes which characterize a lesion and knowledge of the biological behavior of the lesion. The behavior of carcinoma *in situ* of the cervix is not fully understood. Histological diagnosis of carcinoma *in situ*, therefore, should be based on sound criteria; at present it is made too frequently. When a pathologist diagnoses cancer, the validity of his reports is tested in the course of time; when he diagnoses carcinoma *in situ* the lesion generally is removed and whether his opinion was scientifically accurate is difficult to check.

The term carcinoma *in situ* indicates cellular changes characteristic of carcinoma, though remaining intraepithelial. To the histologist, intrinsic cellular change specific to carcinoma is difficult if not impossible to define; the features of carcinoma are recognized in a field of epithelium and concern the architecture of adjacent cells forming a portion of tissue. Even when satisfied that the change present is carcinomatous it is essential to study its relation to adjacent tissue before deciding whether the lesion can be described as *in situ* or otherwise. The first criterion, therefore, is adequate tissue for examination; without this, any diagnosis is incomplete.

The characteristic feature of cervical carcinoma *in situ* is an area of glycogen-deficient squamous epithelium several cells thick with no stratification and comprising crowded, immature, irregular cells. This abnormal epithelium stains deeply with hematoxylin due to the fact that the nuclei are comparatively large and cytoplasm scanty; the chromatin content of the nuclei is coarse or granular and mitoses often abundant. These plump immature cells extend through the thickness of the epithelium and may be loose on the surface. This

pleomorphic picture, in appearance like a segment of anaplastic squamous-cell carcinoma, is usual, but a more uniform pattern is not infrequent. In this the cells are oval or spindle, similar in size, have little or no cytoplasm, the nuclei stain deeply and the number of mitoses is variable; the uniformity of cells may be misleading but the picture resembles a segment of spindle-cell carcinoma of the cervix.

Opinion differs as to whether or not the carcinoma-like changes described should be called carcinoma *in situ* unless the whole thickness of the epithelium is replaced by abnormal cells. This criterion has been over-emphasized; in most cases unless the whole thickness of epithelium is involved the changes present do not merit a designation of carcinoma. On the other hand, a thin film of flattened cells occasionally is seen on the surface of what otherwise is typical carcinoma *in situ*; the problem usually is resolved when there is sufficient tissue for study. In further areas of the same or additional sections typical replacement of the whole epithelium will generally be found to satisfy diagnosis. Cell nests are hardly ever seen while the carcinoma remains intraepithelial.

A common feature is for surface carcinoma to extend into mucous glands and replace the columnar epithelium. When continuity with the surface is apparent it is evident that this glandular involvement is an extension and not an invasion. When the plane of the section fails to show the continuity, care must be taken not to mistake the islands of abnormal epithelium for foci of invasive carcinoma. The anatomical positioning and architecture of the involved glands are often recognizable and there may be strips of remaining columnar epithelium; the rounded outlines are clearly demarcated from the cervical stroma. In cases of doubt it is necessary to cut deeper sections. There is little evidence to attach undue significance to glandular involvement.

The question of stromal invasion may arise from carcinoma *in situ* on the free surface or within the glands. Satisfactory demonstration of a basement membrane beneath the stratified epithelium of the cervix is difficult; in many pathological states cells extend freely from the basal zone into the underyling stroma. Carcinona *in situ* usually is well demarcated from the underlying stroma and in fact has a characteristic tendency to strip from it; not rarely, however, the abnormal epithelial cells extend into the underlying stroma. When the epithelial changes suggest carcinoma, any stromal infiltration must be analyzed carefully but a diagnosis of invasive carcinoma should not be made unless there are obviously proliferating foci of carcinoma quite independent of the surface lesion; individual cells do not satisfy this criterion.

Discussion

Hans F. Bettinger, Melbourne, Australia: There is general agreement among the contributors that carcinoma *in situ* is a lesion which is composed predominantly of quite immature cells. There is some disagreement among the contributors and still far greater general disagreement about the question, how much differentiation in this epithelium is permissible if the lesion is still to be called a carcinoma *in situ*.

Doctors de Brux and Taylor place very narrow limits on this differentiation, allowing only for an imperfect maturation of the two or three uppermost layers. I personally am quite in agreement with these criteria. I think Bajardi is too liberal and the term differentiated carcinoma *in situ* is to me a contradiction in itself. It might be mentioned in passing that I would call none of the lesions that Limburg depicts in a later section carcinoma *in situ*.

There is a very interesting difference of opinion in the papers of de Brux and Taylor, and this concerns the basal membrane. According to de Brux, the "intact basal membrane is perfectly outlined, if not slightly accentuated and sometimes even hyalinized." Taylor says in his last para-

graph: "Satisfactory demonstration of a basement membrane beneath the stratified epithelium of the cervix is difficult: in many pathological states cells extend freely from the basal zone into the underlying stroma." This is not just a personal difference of opinion, for both represent large numbers of pathologists who either believe in the importance of the basal membrane for the diagnosis of an invasive carcinoma or do not. I belong to the latter group, and I wholeheartedly agree with the arguments in Taylor's last paragraph. The results of Siegler's inquiry were probably not unexpected; however, in the legend to Figure 3, he raises a most important point when he asks: "How is questionable early invasion treated at your institution?" The right answer will be found if there is a close cooperation between gynecologist and pathologist, but this is not always the case, and I have seen patients who on the basis of such a questionable finding received full treatment by radiotherapy as well as surgery.

Jorge Campos R. de C., Lima, Peru: The experience of the pathologist is probably the most important factor in the diagnosis of carcinoma *in situ*. Its boundaries, with the early infiltrating carcinoma on one side and with atypical metaplasia on the other side, are not clear. Nevertheless, there is a basic agreement about what kind of structure we call carcinoma *in situ*; Figures 1 and 2 of Bajardi, 1, 2, and 3 of de Brux and Dupré-Froment, and 1 and 2 of Siegler probably do not offer difficulties in the diagnosis for most of the pathologists.

The problem begins when we have a slide similar to Figures 3, 5 or 6 of Siegler. In our experience, a great part of the problem is solved if we follow the criteria mentioned by Bajardi, that is, to do step serial sections of a conization. In the end there are always a few cases with uncertain diagnoses which must be put in a special group in order to study their future evolution.

Marcel Gaudefroy, Lille, Nord, France: All the criteria of carcinoma *in situ* described by Bajardi, de Brux and Dupré-Froment, Siegler and Taylor are classical and commonly accepted The true difficulty is to say if the epithelial changes observed in such cases of atypical epithelium correspond exactly to these criteria. In my opinion, the diagnosis of carcinoma *in situ* must be made only when the pathologist sees all the criteria. The most important features are the abnormal mitoses and the replacement by abnormal cells throughout the entire thickness of the epithelium. When those two conditions are not met I do not speak of carcinoma *in situ*, but only of irregular dysplasia or undifferentiated metaplasia.

Wolfgang Korte, Bonn, Germany: According to most examiners the so-called "carcinoma *in situ*" represents a change which takes place intraepithelially, and is marked by atypias of cells and tissues. The atypias of the cellular patterns and tissue patterns are closely related to the generally accepted criteria for true carcinoma. Invasion is absent. Thus, the decisive histological criterion of the true malignant neoplasm is absent. For any histomorphological definition the clinical course will not be important. A true carcinoma is always a malignant neoplasm in the histological diagnosis, even if it is known that it can be removed by suitable therapy. A so-called carcinoma *in situ* is not defined by the fact that one knows or believes that from it a malignant tumor might arise.

Thus, the designation "carcinoma *in situ*" puts the question to the histopathologist as to which criteria he considers obligatorily for true carcinoma. A number of clinical viewpoints result from the histological diagnosis. The second question is inevitable: Is the carcinoma *in situ* a true carcinoma or not?

It is my opinion that one should not speak of a carcinoma *in situ*. One should at least say that it is a question of a so-called carcinoma *in situ*. It is better to add that it is not a true carcinoma. If one believes that such a declaration might be misunderstood, then one has to point to a potential malignancy. I think that it is too simple and too dangerous for the therapeutic consequences if one defines by the term "carcinoma *in situ*," a tissue change as cancer, although it is not a true carcinoma. It may be that the term is more familiar to the clinician in America and is understood there as the expert histologist would want it to be understood. The reports of Siegler, however, seem to discourage this opinion.

Finally, the orthodox pathologist understands by "surface carcinoma" quite another entity than carcinoma *in situ*. Surface carcinomas are real malignant epitheliomas, which, starting from the surface epithelium, are mainly spread out over the surface of organs, but also show infiltrative growth as every true carcinoma does. I think for this reason that it is hardly suitable to identify a "surface carcinoma," in gynecological lesions, with the so-called carcinoma *in situ*.

Raimund Krimmenau, Dresden, Germany: Medical diagnostic procedures are always analogical conclusions. It is impossible to try to prove mathematically the biological potency of carcinoma *in situ*. The investigated portion is removed and cannot be followed throughout its course. The remaining portion is not examined histologically and consequently is not known. An invasive carcinoma which is found after the histological diagnosis of carcinoma *in situ* can be theoretically assumed as having existed at that time or having arisen independently of the carcinoma *in situ*. The frequency of invasive sections with carcinoma *in situ* justifies, in our opinion, the statement that carcinoma *in situ* and invasive carcinoma belong to the same entity.

The unanimous demand made by the main authors for a tissue specimen in order to permit a conclusion is impressive. From a section alone (unfortunately also from a photomicrograph) no

definite diagnosis is possible, since in the differential diagnoses metaplasia and inflammatory changes are too frequent. Conization alone complies with this diagnostic demand. It is astonishing that the difference between a ring biopsy, cervical amputation and conization, in general, is not at all understood.

The classical picture of Schauenstein surely does not fully apply to all carcinomas *in situ*.

Since Hinselmann's endeavors, a strikingly uniform picture of the appearance and biological potency of carcinoma *in situ*, as well as its differential diagnosis, has originated from various research groups who really deal with it clinically, colposcopically, cytologically, histologically, and prognostically.

The entirely obscure opinions still brought forward nowadays usually originate from authors who apparently have made no survey of the complete features of the entity. Morphological diagnosis is just the same as in the rest of medical science, namely an "interpretation" developed by experience, a fact which is still partially overlooked by some clinicians and morphologists.

F. A. Langley, Manchester, England, U.K.: On reading these accounts of the histological features of carcinoma *in situ* one is struck by the almost unanimity of opinion. Yet, if one pathologist sends a difficult section (not necessarily of carcinoma *in situ*) to several pathologists for an opinion he is quite likely to receive a number of different descriptions of the lesion as well as different diagnoses. Siegler's experiences demonstrate this and emphasize the desirability of some organized exchange of slides and opinions. It must, however, be stressed that the final test of the significance of our criteria is the behavior of the lesion in the patient. Thus, any organized effort to establish diagnostic criteria must correlate histological and clinical findings.

While the importance of abnormal mitoses in these lesions has been referred to, I do not think it has been stressed as much as it might. In a doubtful lesion, especially where it affects glands principally, I lay stress on the occurrence of abnormal mitoses as described by Moricard and Papatheodorous (Gynécologie Obstétrique. **52:** 251, 1953).

Hans Limburg, Homburg, Saar, Germany: I agree entirely in classifying the degrees of maturation of carcinoma *in situ* into immature, moderately differentiated, and differentiated forms. I have used the identical classification previously and also elsewhere in this Symposium. I agree with de Brux in his position regarding the undifferentiated form. I miss, however, mention of the higher differentiated forms of carcinoma *in situ*. The diagnostic results found in the report by Siegler are quite remarkable. One may very well join the opinion of the majority of pathologists regarding the first four Figures, provided that one recognizes the degree of uncertainty resulting from diagnoses from microphotographs. For the same reason I would classify Figures 5 and 6 not as carcinoma *in situ*, but rather as suspicious or observation cases.

Rodolfo Sammartino, Buenos Aires, Argentina: In general I agree with the descriptions presented here regarding carcinoma *in situ*. We must consider it always as an evolutionary process—hence we often encounter intermediate or transitional changes from a beginning basal hyperplasia to an early invasive carcinoma. Consequently, the histological picture can never be fixed and constant for each case and the diagnosis will never be certain or absolute in all cases.

Carcinoma *in situ* is primarily a qualitative question, i.e., first the appearance of a new atypical species of cells, and secondly a quantitative question. The essential thing is the appearance of a cancer-like growth denoting a possible or potential malignancy. Whether or not this real mutation extends to the entire thickness of the epithelium is, in my opinion, a secondary question. For example, (1) is the lateral propagation of a genuine carcinoma *in situ* that which appears as a thin atypical layer against the basal membrane or, (2) are very atypical changes, which to this point have only reached the middle of the epithelium, less worthy to be designated as a carcinoma *in situ*?

I believe that the behavior of the hyaline basal membrane scarcely aids in the diagnosis of invasive cancer. It is often disassociated by exudation or thickened by chronic inflammation, whereas carcinoma, mostly advancing as large buds, tends to push the entire basal membrane against the stroma without destroying it.

John J. Sullivan, Auckland, New Zealand: A discussion on the histomorphology of carcinoma *in situ* is best made from the standpoint of the following definition which embodies our concepts of this disease, i.e., carcinoma *in situ* is of itself an irreversible neoplastic growth, characterized by the presence of a full thickness intraepithelial neoplastic change which may or may not involve gland crypts and which may histologically appear as a pattern of circumscribed ovoid or round collections of neoplastic cells in the stroma of the cervix.

In so far as this definition stresses the importance of a histological pattern in carcinoma *in situ*, rather than the nebulous basement membrane which is or is not intact, there is disagreement with de Brux and Dupré-Froment on the importance which these authors attach to the basement membrane.

To these authors we would pose the following questions:

1. How do you classify lesions in which there is an apparent breach of the basement membrane by one or two or three cells?
2. How many cells need you identify outside a basement membrane before making the diagnosis of invasive carcinoma?

We are of the opinion that some tendency to surface "differentiation" is not in conflict with the concept of a "full thickness intraepithelial neoplastic change" when these surface cells are of the dyskaryotic or neoplastic variety.

While appreciating the precise cytologic divisions which Bajardi has for carcinoma *in situ*, we have not subscribed to further subdivision of this lesion on the grounds that histological classification is only of value provided it

(1) assists the clinician in his interpretation and therapy of disease, or
(2) leads to a better understanding of the nature of disease.

We are in agreement with the observations of Siegler and Taylor who stress the importance of the tissue pattern of carcinoma *in situ*. Further elaboration of the question of cell nests in association with carcinoma *in situ* would be of value in assisting with the interpretation of difficult lesions.

We regard, for the purposes of classification and therapy, all controversial lesions as carcinoma *in situ* until one can demonstrate an unequivocally invasive pattern of neoplasia.

In reply to the question of Siegler, our cases of questionable early invasion are treated by simple hysterectomy and are not classified as cases of invasive carcinoma.

Jaroslav Švejda, Brno, Czechoslovakia: It is rather difficult to state a histological definition of carcinoma *in situ* because the changes which we normally consider as being typical for neoplastic growth are in this case limited to the epithelial layer and one of the most important signs of a malignant process is missing, namely the infiltration into the connective tissue. To a certain degree the evaluation will be subjective and no generally valid definition can be made. One can only try to outline the concept of "carcinoma *in situ*." One may understand as carcinoma *in situ* only such proliferation of cells as that which (1) is evident in all layers of the epithelium, (2) offers the picture of a cellular atypia, (3) exhibits a clear hyperchromasia of the nuclei and (4) exhibits mitoses in many of the cells. The cells contain small amounts of cytoplasm, so that there is a prevalence of nuclei, whereas tonofibrils are not evident. This cellular proliferation does not penetrate the basal membrane in any of the serial sections which are made, not only from the atypical area, but also from the surrounding epithelium. No strict conclusion can be made from the histological picture as far as the biological activity of this atypical proliferation is concerned. In agreement with the clinician it is better to consider it a serious disease and to treat it as such rather than to underestimate it.

Rudolf Ulm, Vienna Austria: Regarding the histomorphology of carcinoma *in situ*, there is no longer any difficulty in its diagnosis among the various authors. In spite of this, the diagnostic field of carcinoma *in situ* is a large one for the pathologist and in the final analysis, the diagnosis "carcinoma *in situ*" depends upon his subjective judgment. As Siegler precisely stated, very different decisions exist in the determination of an already invasive carcinoma. As long as we do not have any routinely recognizable specific signs for the biological evaluation of a suspicious epithelial area, we still depend upon general morphological changes, which are usually found in cases of invasive carcinoma. Our diagnosis of carcinoma *in situ* is, therefore, based on an analogical conclusion. Thus, it follows that the same morphological epithelial changes can be expected to have the same biological potential. Since we should only call those epithelial changes "carcinoma *in situ*" which represent an irreversible criterion, it is, as the main authors emphasize, absolutely necessary to keep the diagnosis of carcinoma *in situ* within narrow limits. For this purpose it would be advantageous to establish general morphological "minimal criteria," i.e., for the most part all layers of the epithelium should consist of immature pathological proliferating cells. These should not tend to develop any maturity towards the surface of the epithelium. Certainly real carcinomas exist, which consist of very mature cell formations. But we cannot recognize such mature cell areas as being carcinomatous in a stage of intraepithelial development and there is no morphological criterion to differentiate them from benign epithelial anomalies.

Just as arbitrary is the determination of the beginning invasion of carcinoma. The examination of the completely existing basal membrane will still remain impossible as long as there exists no possibility for making it completely visible. As a result, the earliest tendency for infiltration of the carcinoma into the connective tissue cannot be recognized morphologically. We therefore consider doubtful invasive epithelial areas as still being intraepithelial formations. We believe that only definite proof of an infiltrative growth into the connective tissue entitles us to make the diagnosis of invasive carcinoma. Such a diagnosis indicates radical surgery or radical irradiation.

Wolfgang Walz, Heidenheim, a.d. Brenz, Germany: The histomorphological descriptions and the appreciation of carcinoma *in situ* by the main authors are rather uniform and we can only agree. I would especially like to support the opinion of Bajardi, that, analogous to invasive carcinoma, there are also various degrees of maturation in carcinoma *in situ*.

As shown by Siegler, however, occasionally there are marked differences in spite of this uniformity of diagnosis. The main difficulty does not appear to be so much in the recognition of beginning invasion but of differentiation towards the dysplasias. Taylor points to the very important fact that there are no specific features known for the single carcinomatous cell. Thus, the diagnosis of

carcinoma *in situ* depends upon the qualitative and quantitative formation of certain characteristics of the single cell and of the entire epithelium as well. Therefore, the diagnosis in borderline cases often depends upon the subjective viewpoint of the individual examiner. In addition to the facts already stressed by the authors, I would like to draw attention to the following points:

1. Alteration of the basal cellular layer; if it is entirely irregular and altered with respect to the overlying layers, then in my opinion, we are dealing with a carcinoma *in situ*. As an example I would like to point to Figure 1 of Bajardi. If, however, the basal cells are situated in palisade-like formations and do not display pronounced polymorphia, then we are dealing with a dysplasia.

2. Within a carcinoma *in situ*, as well as within a dysplasia only rare inflammatory cells are found. This seems important to us for differentiating this state from an epithelium initiated by inflammation.

3. A so-called basal membrane does not exist. I refer to the electron microscope investigations of Albertini and Glatthaar (Oncologia. **8:** 2, 1955).

Antoine Zajdela, Paris, France: We agree with the definition, and all the criteria, of carcinoma *in situ* which have been described by the main authors.

However, if carcinoma *in situ* would seem well defined theoretically, nevertheless in practice the problem remains, for even when all the various criteria of intraepithelial malignancy may be found together (which seldom is the case), each pathologist may have his own interpretation and the interpretation varies according to the investigator. With regard to this, Siegler's paper is conclusive.

On the other hand, the pathologist is often asked to give his initial opinion on a biopsy which is sometimes only a partial picture of the lesion. Moreover, as Bajardi has rightly stated, a carcinoma which looks intraepithelial in one area may be invasive in another area. The diagnosis of a carcinoma *in situ* is, therefore, only valid after a thorough examination of multiple sections of the entire cervix. But the investigation of uterine cervices by multiple sections leads to difficult practical problems, more especially since we would have to use our entire stock of labeled blocks in serial section studies.

Nevertheless, according to Gricouroff (Pres. Med. **34:** 713, 1952), if the cytomorphological picture of the carcinoma *in situ* is at present well-known, it does not follow that the lesion is always malignant. The same picture may correspond either to a surface carcinoma, or to a dysplasia morphologically alarming, but which is not a carcinoma *in situ* and must not be considered as necessarily leading to a carcinoma. (In this connection, I should insist on the difficulty of diagnosis during pregnancy, when the cervix is modified by the hormonal status and inflammation and gives a picture which might easily be mistaken for that of a carcinoma *in situ*.)

According to Siegler's methods, I think that all material suspected of being a carcinoma *in situ* should be reviewed and interpreted by several pathologists. Their opinions would lead to a more definite acquaintance with the case and to a far more valid diagnosis.

Closing Remarks

Edward E. Siegler: 1. Cervical carcinoma *in situ* frequently remains a more controversial histological diagnosis than other areas of histopathology, even after step-sectioning and even after years of experience, since experience in untreated carcinoma *in situ* is the only worthwhile teacher.

2. With our present state of knowledge, the only plan I know which might be helpful would be the following: Perform only small punch biopsies on patients with positive cytological smears of the type consistent with carcinoma *in situ*. Using the cytological-histological correlation technic, such as demonstrated in a later section of this Symposium, one can frequently be satisfied that the entire cellular pattern of the smear has been explained by the histological changes. Sometime after healing (in a few months), repeat the smears and if the second set resembles the first, one can assume that the lesion has not been completely removed. Every six months thereafter, for at least 15 years, repeat the smears, examine the cervix and occasionally remove a small piece of tissue. Eventually, you will learn which of the patterns progress, which remain stationary, and which disappear. (It must be admitted that even this procedure is not infallible.)

3. I agree with Korte that the term carcinoma *in situ* is a poor term and should be abandoned, although, because of common usage, it probably never will be. To use a term which means a malignant benign tumor should not be acceptable to our scientific principles. To use a term such as "possibly pre-malignant" is far more the truth.

4. Until the time arrives in the future when we know which of the patterns we now interpret as carcinoma *in situ* are really preinvasive carcinomas, then I believe like Švejda does that total hysterectomy is the treatment of choice.

5. Word descriptions are not adequate enough to cover all of the many variations in the histological patterns of carcinoma *in situ*. Exchange of controversial slides is the only median of worthwhile discussions—some central organization should act as registry.

Carlo Sirtori: I think that the observations I made about the atypical non-invasive areas surrounding the invasive carcinomas are worth a histomorphological diagnosis of carcinoma *in situ*. I also believe it is more than right to see several sections. I usually advise, in doubtful cases, to repeat the biopsy under colposcopical guidance. In order to avoid terminological misunderstandings I think it is better to keep, for carcinoma *in situ*, the description used up to the present time, i.e.,

carcinoma *in situ* means presence of atypical cells throughout the thickness of the epithelium; these atypical cells must be disposed irregularly and present the so-called self-invasion phenomenon. In short, carcinoma *in situ* means an epithelium where it is impossible to distinguish the basal from the surface cells because they show the same pattern of nuclear hyperchromasia and atypia. Mitoses will be more frequent and atypical and, above all, will be present throughout the extent of the epithelium. In fact, the most important difference is the following: in the normal squamous epithelium the germinative layer is located near the basal membrane, whereas, carcinoma *in situ* is completely formed by cell types of the germinative layers. Finally, the carcinoma *in situ* is characterized by the presence of the basal membrane; if the latter is not present, we speak of invasive cancer.

As far as the presence of differentiated cells in carcinoma *in situ* is concerned, I think that for practical purposes it is better to classify as "carcinoma *in situ*" only the proliferative forms without differentiated elements.

Claud W. Taylor: In general there seems to be agreement that carcinoma *in situ* characteristically is composed of immature epithelial cells; a lesion showing maturation of any but the most superficial cells probably should not be classified as carcinoma *in situ*. The remarks of Korte regarding this designation are worthy of special note.

Difficulties in diagnosis in the main seem to result from the examination of insufficient tissue. Small biopsies cause pathologists often to give only provisional reports which are then bound to vary from pathologist to pathologist; when adequate tissue is available for study these differences of opinion largely disappear.

What Constitutes a Definite Diagnosis of Carcinoma *In Situ* with Special Reference to the Amount of Histological Sections Required ("Serial Sections") and Type of Material?

JORGE CAMPOS R. DE C.
Lima, Peru

DESPITE the fact that cytology is a great help in discovering subclinical cervical carcinomas, the diagnosis of cancer *in situ* is finally always a histological procedure.

The routine of our laboratory in establishing a definitive diagnosis of cervical carcinoma *in situ* is as follows: when by a cytological smear and/or by a cervical biopsy, a carcinoma with the histological characteristics of carcinoma *in situ* is discovered in a woman who, clinically, was not suspected to have cancer, an annular cervical biopsy is made comprising the internal part of the ectocervix, the lower part of the endocervix and the junction line of both epithelia. This resection is always made with a cold knife and followed by electrical coagulation.

The cervical ring specimen thus obtained is cut radially, taking eight samples, each one of them comprising the ectocervix and endocervix. Each sample is embedded separately in paraffin and three or four sections are taken at different levels providing 28 or 30 slides for examination.

Besides the number of slides studied, it is important to have definite histological criteria to diagnose carcinoma *in situ*, because this lesion has a rather characteristic histology, as is well known.

It is enough to find invasion in one field in order to discard the diagnosis of carcinoma *in situ*. The spread of the lesion to the endocervical glands does not invalidate the diagnosis of carcinoma *in situ*. In doubtful cases where there are some fields suggestive of invasion, we prefer to call them early invasive carcinomas.

Summarizing, we do not establish a diagnosis of carcinoma *in situ* based on one histological section nor on "serial" sections of one isolated cervical biopsy.

EDMUND SCHÜLLER
Vienna, Austria

THE definite diagnosis of a carcinoma *in situ* of the uterine cervix presupposes that one has the whole lesion surrounded by healthy tissue in one's specimen. Therefore, only material obtained by amputation or by cone biopsy of the cervix is sufficient for a definite diagnosis. This material must be completely worked up in several blocks. Smaller cones can be cut median sagittally in two parts or for larger specimens one can begin at 12 o'clock in clockwise direction and cut into several numbered pieces. For detection of an early invasion, it is not necessary to perform serial sections. It is sufficient to make, from every block 12 to 20 sections for a distance of about 200 micra. Some gynecological pathologists have asserted that they found an invasive carcinoma only after 1,500 serial sections. Such a small area of "invasion" has certainly no importance, in my opinion.

With this definite diagnostic procedure one has also done sufficient therapy in cases of carcinoma *colli uteri in situ*.

CLAUD W. TAYLOR
Birmingham, England, U.K.

CARCINOMA *in situ* not infrequently is found in association with established cervical carcinoma. When carcinoma *in situ* is detected in a portion of cervical tissue it is thus imperative to exclude the presence of coexistent invasive carcinoma elsewhere in the cervix. Carcinoma *in situ* takes origin near the squamo-columnar junction in more than one place; the lesion may spread over the portio and along the endocervix. Progression to invasive carcinoma may occur in any of the affected areas. For these various reasons an unqualified diagnosis of carcinoma *in situ* can be made with certainty only when the whole cervix is available for examination. In practice this standard of diagnosis is neither practical nor necessary.

Diagnostic biopsy usually is undertaken due to cytological findings; the cervix may show no clinical lesion to indicate biopsy site. The selection of biopsy site and amount of tissue to be removed depends upon the age, parity and clinical history of the patient and the facilities available. Affected areas may be ascertained by iodine staining or colposcopy and removed for histological study; small biopsies carefully selected in this way can be representative. In the absence of such selection quadrant biopsies can be reasonably satisfactory but a ring biopsy including the whole squamo-columnar junction is preferable. In any of these methods the biopsy must be deep enough to show the relation of the epithelium to the underlying cervical stroma and at the same time the endocervical mucosa should be curetted to ascertain whether or not the lesion is present within the cervical canal. Information gained from these procedures is often sufficient to warrant a firm diagnosis of carcinoma *in situ* but must be followed by careful cytological follow up and repeat biopsies where indicated.

When possible conization of the cervix to yield a larger portion of tissue is desirable. In many instances such a specimen allows complete study of the lesion; not only can progression to or association with invasive carcinoma be found or excluded but the limitations of spread of the *in situ*

change can be fully determined. There are many technical difficulties in obtaining and preparing a satisfactory ring biopsy for histological study—the ring often is deficient in some areas and examination therefore incomplete. Conization is really an extension of the ring biopsy and to the histologist is preferable. The information obtained may be complete in itself and if not will indicate the need for further excision.

A biopsy specimen of any type should be cut into segments a few millimeters in thickness with the undisturbed epithelial surface forming one border—the whole of the surface epithelium of each segment is then available for study together with the underlying stroma. Segments properly prepared will block out flat in paraffin and yield a representative section on cutting; if serial or step sections are not routinely cut the paraffin block should remain on its block holder so that further sections may be taken consecutively as required. If the paraffin block has to be reset, sections will be lost on the recut.

A representative section from each thin segment of a conization specimen gives a reasonably complete picture of the histology of the cervix—no established carcinoma will be missed. If there is doubt regarding invasion or about the *in situ* lesion then deeper sections can be cut from the respective blocks. Routine serial sections, though desirable for research, are beyond the scope of many laboratories; about every tenth section may be mounted and stained. It is doubtful if a significant lesion is missed by the routine suggested providing the deeper step sections are cut in any case of doubt.

Carcinoma *in situ* should be diagnosed on sound histological criteria—the diagnosis refers only to the portion of tissue examined. In practice a conization specimen, cut into many thin segments from each of which a representative section can be obtained, offers sufficient tissue for a definite diagnosis of carcinoma *in situ* of the cervix. Multiple sections are desirable when practicable but laboratories without these facilities can give adequate diagnoses.

Discussion

Anthony F. Anderson, Edinburgh, Scotland, U.K.: We agree with Campos and Schüller. Taylor seems to me still to be having growing pains; at the end of his first paragraph he states such a standard as the whole cervix is neither practical nor necessary. I believe it is—or the cone biopsy he finally reaches in his third paragraph—when the reason for biopsy is positive cytology and the clinician cannot *see* the positive area. The selection of biopsy site depends on only one thing to my mind, whether the clinician can see a cancerous area or not. If he can he takes a biopsy from it, if he can't he must take a cone or the whole cervix. This is not a high standard. Curettage of the endocervix may be entirely misleading, carrying no information about invasion (unless we change to adenocarcinoma of the body of the uterus). At the end he does appear to be another pathologist "gagne."

Friedrich Bajardi, Graz, Austria: We agree with Schüller that for the safe histological diagnosis of a carcinoma *in situ* there has to be a specimen on hand in which the entire lesion is surrounded by healthy tissue. We consider conization of the cervix the proper procedure for obtaining a specimen of this kind. The procedure is performed with the aid of the colposcopic picture and the iodine test and is performed in such a way that the entire altered area of the ectocervix is excised within healthy tissue, as is proposed also by Taylor. In order to also include endocervical lesions, we take pains to extend the cone as close to the isthmus as possible. In order not to disturb the histological picture, we curette the remaining part of the endocervix only after the biopsy is taken.

After fixation, the cone is divided into a right and a left portion by cutting through the longitudinal axis of the endocervical canal. Both parts are embedded in paraffin and cut. With this procedure it is possible to survey the entire epithelium of the cervix, beginning with the normal squamous epithelium of the ectocervix through the pathological epithelium and up to the cylindrical epithelium of the endocervix.

In agreement with the main speakers we do not believe that for the detection of a microinvasion, serial sections are always necessary. In the majority of cases a reasonable number of sections cut from the various portions of the specimen

should suffice. However, for our own investigation and predominantly for scientific reasons we do semi-serial sections at intervals of 100 micra.

Hans F. Bettinger, Melbourne, Australia: I agree with the three contributors that serial sections are not only impracticable except in research laboratories, but that they are also not necessary if the methods outlined by the authors are followed.

James Henry Ferguson and H. Schulman, Miami, Florida: We emphatically agree with Schüller and Taylor that a large specimen of the cervix is necessary in order to diagnose confidently carcinoma *in situ*. The entire cervix or, more practically, a large cone specimen, must be adequately studied. Selected portions of the cervix, obtained by a punch biopsy or a knife, and even the ring specimen, are unsatisfactory because an area of invasion in the cervix can be missed. Our comparisons of punch and cone specimens demonstrate the need for conization when there is positive cytology and a tissue diagnosis less serious than invasive carcinoma.[1, 2] The unacceptability of a punch biopsy diagnosis of carcinoma *in situ* is demonstrated in our series of 87 women who had conizations following punch biopsy: 17 (20 per cent) of the cone specimens showed invasion.

Dr. Schüller states that diagnostic conization is sufficient therapy for carcinoma *in situ*, but our experience does not permit such confidence. Eighty-four women had that diagnosis from a cone specimen and subsequently had a hysterectomy; 14 still had carcinoma *in situ* in the uterus and 12 had various degrees of dysplasia.[2] The disease remaining in the uterus cannot be explained on the basis of removal of a scanty amount of tissue at conization.[3]

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Clarice do Amaral Ferreira, Rio de Janeiro, Brazil: The management used in the Instituto de Ginecologia da Universidade do Brasil (Rio de Janeiro), according to the orientation of Professor Arnaldo de Moraes and dealing with the definite diagnosis of carcinoma *in situ*, is approximately that used by Schüller and Taylor. It is as follows:

a. In those patients on whom cytology and colposcopy have indicated the possible occurrence of cancer, a selective biopsy is made, which means biopsy under guidance of colposcopic control.

b. If carcinoma *in situ* is the diagnosis made by the pathologist, a low cervical amputation is carried out and serial histological sections are made; the number of blocks prepared for embedding from each cervix depends on the size of the specimen.

c. If this histological examination confirms that the lesion is a real carcinoma *in situ*, the patient will be followed by cytology and colposcopy. If she has some associated gynecologic disease, as for example, a myomatous uterus, a simple, total hysterectomy is carried out. If invasion is found, she is submitted to a complete hysterectomy, with lymphadenectomy.

The pathologist of the Instituto de Ginecologia, Nisio M. Fonseca and Sebastiao Coutinho do insist on some points:

1. Diathermic biopsy or electro-cauterization after cold conization is contraindicated, before or after the definite diagnosis.

2. The number of blocks in the histologic examination of the biopsy specimen depends upon the size of the extirpated tissue; four or more blocks are usually prepared.

3. The number of serial sections obtained also depends upon the size of the cervix; one section is stained out of every ten sectioned.

4. It is essential that the histological examination includes all of the borders of the lesion in order to know if enough normal tissue surrounds all of the pathological epithelial lesion.

5. Any kind of invasion, even early, will classify the case as invasive cancer.

Emmerich von Haam, Columbus, Ohio: I agree with the authors of this Symposium that step serial sections taken through the six or eight blocks prepared from a good cervical ring biopsy or ring conus are sufficient to detect not only carcinoma *in situ* but also to rule out invasive carcinoma. We also recommend continuing the cytological studies after the cervical conization has been taken and the wound has healed (two weeks) in order to be sure that nothing remains.

Hans-Ludvig Kottmeier, Stockholm, Sweden: What constitutes a definite diagnosis of carcinoma *in situ* with special reference to the amount of histological sections required and the type of material required?

On suspicion of carcinoma *in situ* in curettings or biopsies, a careful examination is necessary. Such an examination includes a conization with excision of the endocervix and removal of those areas on the portio which, by Schiller's iodine test, seem to contain little glycogen.

The cone is perpendicularly cut into twelve pieces and the pieces are registered. Four to five slides are examined from each piece. Provided a questionable invasive carcinoma is observed in one or several of these pieces, semi-serial sectioning is performed on these blocks. This histological routine examination has revealed an obviously invasive carcinoma in 20 per cent of the cases suspicious of carcinoma *in situ*, which, clinically, were not suspected of having carcinoma.

Schüller writes: "With this definite diagnostic procedure you have also done sufficient therapy in cases of carcinoma *in situ*." We do agree that a

gynecologist is permitted to rely on the conization, provided histological examination has made it likely that the anaplastic epithelium has been completely removed, and furthermore that the patient is willing to undergo regular follow up examinations. Although histological examination has made it likely that the carcinoma *in situ* had been completely removed by the conization, an invasive carcinoma occurred later in five of 107 cases, in which the conization was performed in the years 1949 to 1956. A careful follow up of patients conservatively treated for carcinoma *in situ* must include the taking of vaginal and cervical smears.

As far as the diagnosis of invasive carcinoma is concerned, Fidler and Boyes have found it of value to define two stages of invasion. Their discussion is of considerable interest. Cases included in the first stage of invasion have been allotted to Stage 0 at the Radiumhemmet. We are well aware of the fact that the interpretation of these early cases varies from one institution to another. This renders a comparison of therapeutic statistics difficult. We would recommend allotting these early cases of invasive carcinoma to Stage I, provided this stage is divided into two sub-groups. Cases of preclinical carcinoma should be referred to as Stage IA.

Ernst-Helmut Krüger, Halle, Saale, Germany: We agree with Campos that the correct diagnosis of carcinoma *in situ* cannot be done from a single section or a few histological sections from a punch biopsy or a cold knife biopsy, both of which are objected to by us. Only removal of the entire suspicious tissue and a work-up of continuous serial sections can establish the diagnosis. One will never miss the important squamo-columnar junction by doing a ring biopsy. The precise area of the atypical epithelium may easily be identified by means of the Schiller test. The vagina and cervix should not be touched before the biopsy. The four-quadrant biopsy is unsatisfactory to us. The idea of a histological examination for clarification of the question, "Is the lesion still a so-called carcinoma *in situ*?" is, after all, to safely exclude the presence of infiltrative growth. In order to demonstrate an early invasion, semi-serial sections are not sufficient, however. We, too, doubt whether or not removal of all parts of carcinoma *in situ* is sufficient therapy. Our experience allows the conclusion that excision of the involved tissue alone is not sufficient and safe therapy. We believe that the generally accepted prognostic evaluation of carcinoma *in situ* and the therapeutic consequences derived from this are due for revision.

Without a screening of the serial sections, one by one, there is no differentiation possible between the so-called carcinoma *in situ* and the early true carcinoma. The specimen from a punch biopsy by no means allows conclusions for a justifiable therapeutic procedure. In the case of a transition into carcinoma the question has to remain open as to whether or not true carcinoma was already present at the time the first biopsy led to the diagnosis of "carcinoma *in situ*," unless the entire suspicious tissue has been examined histologically.

Within the period of six and one-half years of the 83,767 women examined for the first time and primarily subjected to colposcopy, 388 (0.46 per cent) had a histological serial section study due to colposcopically suspicious uterine cervix. This corresponds to a serial section histological examination for every 215 women examined for the first time. Ninety minimally invasive carcinomas were found, among which were 50 so-called "micro-carcinomas" and 126 so-called "carcinomas *in situ*" (32.4 per cent of the suspicious cases and 1:664 for the whole series). In order to ascertain the diagnosis of "carcinoma *in situ*" or micro-carcinoma on the average, 990 histological sections were necessary. The results show that predicting the histological finding from the colposcopical picture is possible to only a limited extent, since 44 per cent of all of our ring biopsies (work-up in serial sections) did not show any malignancy. The results also reveal that the finding of an atypical epithelium on the first colposcopical examination will then result in the additional finding of carcinoma *in situ* in 32 per cent of the cases. Serial sections are tedious, troublesome, time-consuming, and expensive. For the definite diagnosis of a so-called carcinoma *in situ*, however, they are indispensible.

F. A. Langley, Manchester, England, U.K.: There seems general agreement among the writers that a ring or cone biopsy is the preparation of choice and that serial section is unnecessary to establish the diagnosis of carcinoma *in situ*. Campos suggests dividing the tissue into eight samples; perhaps he does not use this number as a fixed rule but rather as a guide. I usually take 12 samples but if the cervical os is small this may not be possible, whereas if it is large and patulous the biopsy may be larger and more samples can be taken. It is well to try to obtain a fairly constant thickness of block for embedding (a few millimeters as Taylor suggests) so that the sample size remains constant. If the samples are small and approximately uniform in thickness, then the method described by Taylor is adequate and the procedure of leaving the tissue on the holder very useful.

One difficulty I encounter from time to time is due to the tendency of the epithelium to strip off the stroma when sectioning, especially if the block is sectioned from stroma to epithelium. Is

there an easy way to orientate the tissue to avoid this?

Hans Limburg, Homburg-Saar, Germany: In our experience, colposcopy, in addition to cytology, has proven to be an invaluable aid in finding by direct visualization with 10x enlargement, the correct site for taking the biopsy. In case a carcinoma *in situ* is found, we feel, as does Taylor, that a conization of the cervix and subsequent examination of every tenth section is better than a ring biopsy, since more tissue is made available. The final histological diagnosis can be made only from the amputated cervix.

In the case of the rare intracervical carcinoma *in situ* colposcopy cannot be effective. Hence, when colposcopy is negative with positive cytology, we perform first of all a cervical curettage. A conization may follow for further histological evaluation is case carcinoma *in situ* has been diagnosed from the curettage.

Rudolf Ulm, Vienna, Austria: We are of the same opinion as the three main speakers. The diagnosis of a carcinoma *in situ* can only be made if the whole suspicious tissue has been cut out in the healthy tissue and has been examined histologically in serial sections. Small biopsies are insufficient. Only a ring biopsy, a conization or an amputation of the cervix is sufficient for the histological examination. A true histological serial section, according to our experience, is not necessary for the routine examination. From the scientific point of view, we have performed complete histological sections of several uterine cervices, and it was found that single sections at distances of approximately 200 micra give as precise information concerning the structure of the tissue as do complete serial sections. In any case, we believe that the beginning invasive processes, which are so small that they are hidden within serial sections at a distance of 200 micra, do not have any practical value, at least not to the extent so as to give suspicion for the existence of metastases.

Closing Remarks

Jorge Campos R. de C.: Langley is correct. When I said that we take eight samples from the cervical ring specimen, it is because it is the most usual number of fractions into which we can divide the whole specimen. The number of fractions is not a fixed one, it depends on the size of the cervical specimen.

I agree entirely with the suggestion of von Haam, when he proposed "continuing the cytological studies after the cervical conization has been taken and the wound has healed (two weeks) in order to be certain that nothing remains." We do the same routine because we are not certain if carcinoma remains, even if the borders of the specimen were free of carcinoma, because it is well-known that carcinoma *in situ* has a non-continuous topography.

Edmund Schüller: Again it has to be emphasized that an extensive conization is only sufficient therapy for certain cases of carcinoma *in situ*. The careful examination of the removed tissue must be made to determine whether or not the lesion has been removed from healthy surroundings. If one cannot give this assurance, a hysterectomy must be performed. One also should insist that after conization, the patient be cytologically observed at regular intervals.

Claud W. Taylor: Adequate tissue for study regarding a diagnosis of carcinoma *in situ* generally is accepted as meaning a conization specimen of the cervix. Only in this way can the true nature, extent and limitations of a lesion be assessed. Several blocks of tissue with step-sections from each form a practical basis for study. Langley's observation regarding the stripping of the surface epithelium raises a very practical problem difficult to answer; carcinoma *in situ* seems to be less adhesive to the stroma than normal stratified epithelium and stripping may be accentuated by swabbing of the cervix at the time of operation.

The Maurice Goldblatt Cytology Award

The Editors of *Acta Cytologica* take great pride in announcing the establishment of the Maurice Goldblatt Cytology Award sponsored by the Cancer Research Foundation of Chicago, Illinois, U.S.A.

Statutes of the Award

Purpose: The Award is given annually to a person or persons who has (or have) made an outstanding contribution to the field of cytology during the past five (5) years. Special consideration is given to publications which have appeared in *Acta Cytologica*, the Journal of Exfoliative Cytology.

Sponsor: The Award is sponsored by the Cancer Research Foundation, Chicago, Illinois, U.S.A. on an annual basis.

Award: The selected person or persons will receive a check or will share in a check in the amount of $1,500 (fifteen hundred U.S. dollars) and will be presented with a medal. If the Award is given to two or more persons who have collaborated, the medal and the check will be presented to the group, and replicas of the medal will be presented to each of the collaborators. The Jury may refrain from making an Award in any year. A nominee who fails to receive the Award may be nominated for the Award for the same work in a subsequent year. No person may receive more than one Award for the same contribution, although he may receive a later Award for a different research project.

Nomination: Any member of the faculty of a medical school and/or member of good standing of a national or international cytological society may nominate a person or persons for the Award.

Jury: The Jury for the Award will consist of the Chairman of the Board of the Cancer Research Foundation, Mr. Maurice Goldblatt as Honorary Chairman, a medical or scientific consultant to be nominated annually by the sponsor and the Members of the Editorial Board of *Acta Cytologica* as Jurors. The Jury will, therefore, consist at this time (March 15, 1961) of the following members:

1. Dr. Clarice do Amaral Ferreira, University of Brazil, Rio de Janeiro, Brazil.
2. Dr. Morris Fishbein, Chicago, Illinois, U.S.A.
3. Dr. Ruth M. Graham, Roswell Park Memorial Institute, Buffalo, New York, U.S.A.
4. Dr. Emmerich von Haam, Ohio State University, Columbus, Ohio, U.S.A.
5. Dr. Leopold G. Koss, Memorial Center for Cancer and Allied Diseases, New York, New York, U.S.A.
6. Dr. George N. Papanicolaou, Cornell University Medical College, New York, New York, U.S.A.
7. Dr. J. Paul Pundel, Maternite, Luxembourg, Luxembourg
8. Dr. James W. Reagan, Western Reserve University, Cleveland, Ohio, U.S.A.
9. Dr. George L. Wied, University of Chicago, Chicago, Illinois, U.S.A.

Material Supporting Nomination: The material supporting the nomination should include:

1. Ten copies of a statement covering the academic history and accomplishments of the nominee;
2. Ten copies of a reasoned statement of the basis for the nomination;
3. Ten copies of reprints reporting the nominee's contributions.

All material supporting a nomination must be in the hands of the Editoral Office of Acta Cytologica, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A. by August 1 of each calendar year.

Book Reviews

Il Cancro del'Utero Dalla Macroscopia all' Ultrastruttura. Carlo Sirtori and Ettore Morano. Milan, Italy, Casa Editrice Ambrosiana Publisher. 1960.

THE monograph in the Italian language by Professor Carlo Sirtori, President of the Carlo Erba Foundation, Milan, Italy, and by Professor Ettore Morano, Director, Tumor Institute, Milan, Italy contains 268 pages with 211 illustrations, most of which are excellent. It is one of the few monographs dealing with uterine carcinoma which presents the various viewpoints, diagnostic procedures and scientific methods of evaluation in one concise, informative and well written volume.

The book is divided into four parts. The first part on carcinoma of the portio vaginalis, comprises half of the book, and deals with diagnostic methods, normal and pathological aspects of the portio as seen by various methods, the morphological evaluation of the radiosensitivity of the carcinoma of the cervix, and histochemical, histophysical, and hormonal investigations. This section contains some of the most outstanding photomicrographs of the many excellent ones in the book.

The second part is on carcinoma of the endocervix and of the endometrium. Then follows part three, on electron microscopy of the epithelium of the portio vaginalis and of the endometrium, and part four, the conclusions and clinical summary of the original part of the work.

This well-illustrated book is comprehensive in its scope and thorough in its approach to the subject of uterine carcinoma. One of the outstanding features is the discussion of clinical diagnostic features such as colposcopy, in addition to routine cytology, and the devotion of an extensive portion to cytological research, cytochemistry, spectrophotometry, interference microscopy, phase microscopy, and electron microscopy. In the opinion of this reviewer, the portion on electron microscopy seems to be the most unique and important addition of the monograph to our current literature on the topic of uterine carcinoma. The monograph fills a real need and should be considered for translation into other languages.—*G. L. W.*

Supplement Two of The Atlas of Exfoliative Cytology. George N. Papanicolaou. Cambridge, Massachusetts, Harvard University Press. 1960.

THE second supplement to The Atlas of Exfoliative Cytology by Professor Papanicolaou contains a new chapter on cancer cells in the circulating blood with the description of the technic for isolation of the malignant cells, and descriptions of nonmalignant and malignant cytology. Three pages of color plates illustrate the findings in the circulating blood. These plates and the description were prepared in collaboration with Elizabeth A. McGrew, M.D.

Other new plates of color photographs contain histological and cytological patterns found in adenocarcinoma of the endometrium and endocervix, in adenocanthoma, in secondary carcinoma of the vagina, in condyloma acuminatum of the cervix, in carcinoma *in situ* of the cervix and papilloma of the vagina.

In the field of cytology of the urinary and male genital systems, the new supplement contains color plates on sarcoma of the penis and intraepithelial carcinoma of the bladder, papillary carcinoma of the renal pelvis and ureter and papillary carcinoma of the bladder. In the field of cytology of the respiratory system the new addition to the Atlas contains color plates on carcinoma of the lung metastatic from the breast and adenocarcinoma of the lung, whereas in the field of cytology of the digestive system one finds new plates on adenocarcinoma of the pancreas, on papilloma and carcinoma of the stomach and on Hodgkin's disease. There are also new plates on cytology of exudates, namely on malignant mesothelioma of the pleura, neurogenic sarcoma and liposarcoma, and finally a plate on malignant cells found in spinal fluid.

The unique loose-leaf form of the Atlas permits easy addition of new pages and plates. Therefore, the Atlas will never be outdated, since new developments in the field of exfoliative cytology may be added at any time. The Atlas of Exfoliative Cytology by Papanicolaou, including this new supplement and those that surely will follow is an absolute must for the clinical cytologist, the cytopathologist, the cytologically interested clinician and the cytotechnologist.—*G. L. W.*

DIAGNOSTIC CYTOLOGY—and Its Histopathologic Bases. Leopold G. Koss, M.D., in association with Grace R. Durfee, B.S. J. B. Lippincott Company, Philadelphia, Pennsylvania, U.S.A., 1961. 380 pages, 776 figures. $16.50.

THE PRESENT TEXT is divided into two main parts: (a) General Cytology, and (b) Diagnostic Cytology of Organs. In the first part of the book the authors describe the Cell, Its Structure and Functions, General Classification of Cells and Tissues, Benign Pathologic Processes Affecting Cells, Morphologic Estimation of Cellular Activity and Death in Fixed Material, and General Pathology and Cytology of Tumors. A list of 50 references concludes this particular section, which is, in the opinion of this reviewer, an important feature of the book. In this section of the book the authors describe the details of general cytology which the cytopathologist and cytotechnologist need to know for the understanding of the intricate diagnostic cytology which follows.

In the second part, devoted to Diagnostic Cytology of Organs, the following individual chapters are found: Basic Principles of Cytologic Diagnosis, Female Genital Tract in the Absence of Cancer, Carcinoma of the Cervix Uteri, Proliferative Disorders and Tumors of the Endometrium, Other Tumors of the Genital Tract, Respiratory Tract, Urinary Tract, Gastro-intestinal Tract, Effusions, Cancer Cells in Circulating Blood, Nipple Secretions in the Nonlactating Breast, Aspiration Biopsy Smear (by Dr. John W. Berg), Cytologic Technics and Some Useful Suggestions for the Proper Operation of a Laboratory of Diagnostic Cytology. A list of usefully classified references of 470 publications concludes the second part of the text.

The outstanding features of the book are the comparative cytological and histological findings, the concise and instructive text, and the numerous and generally excellent photomicrographs. In view of this most important new addition to cytological literature, it might sound petty for this reviewer to point out that the authors still use the term "precornified cells" for the type of cells one would call "intermediate" according to the new terminology suggested in No. 1 of Volume II of *Acta Cytologica*. The same holds true for their usage of the terms "cornified cells" instead of "superficial cells" and "basophilic-acidophilic" instead of "cyanophilic-eosinophilic," voted upon by the majority of the cytologists participating in the Terminology Symposium. However, this distracts in no way from the prominence and importance of this excellent book.

It is the sincere feeling of this reviewer that no cytopathologist and no cytology laboratory, as well as no cytologically interested clinician should be without this book. The publishers should also be congratulated for the excellent composition and illustrations (black and white as well as colored photographs). Translations into other languages should be seriously considered in the near future.

Dr. Leopold G. Koss (Attending Pathologist, Memorial Center for Cancer and Allied Diseases, New York City; Head, Section of Cytopathology; Associate Professor of Pathology, Cornell University), in association with Grace R. Durfee, B.S. (Chief-Cytotechnologist of the Memorial Center for Cancer and Allied Diseases), has given us a needed, and lasting addition to our cytological literature with this important book.—*G. L. W.*

Abstracts

The significance of the biological readiness of the organism for the induction of labor in protracted pregnancies. Židovsky, Jan and Jiří Ulřych: Gynecologie et Obstetrique. 259: 1960.

IN the present paper attention is drawn to the importance of the biological readiness of the organism during protracted pregnancy, before the contemplated induction of labor. This should be ascertained by means of vaginal cytology and palpation of the uterine cervix. An analysis is presented of all protracted pregnancies recorded in 1957-58. The results of induction and medicamentous sensibilization are discussed, with regard to the cytodiagnostic and palpation criteria of the readiness of the organism for labor and their simultaneous evaluation for the expected result of induction of labor. The procedure, particularly the time schedule of induction of labor in physiological protracted pregnancies, is defined.

Vaginal cytology, in combination with palpa-

tion of the uterine cervix, is one of our best methods of judging the biological readiness for delivery of pregnant women.

Thus maximal success is insured with induction of labor from the point of view of time and method. Using both examinations simultaneously it is possible to eliminate unnecessary inductions, and lower the number of dystocias and operative deliveries.

Out of 173 inductions of labor omitting 36 cases of drug-sensitivity we succeeded in 98.9% using the evaluation of both methods simultaneously.

Contribution to the cytodiagnosis of the delivery date in prolonged pregnancy. Židovsky, Jan: Zentralblatt für Gynäkologie. **82:** 513-523, 1960.

THE dynamic changes of the vaginal squamous epithelium in smears during the last months of pregnancy are presented.

According to those changes it is possible to predict the delivery date. The cytodiagnostic criteria for the proper delivery date were categorized into four patterns.

During the past five years investigations were made by means of vaginal cytology on 427 pregnant women with prolonged pregnancy. In 76 women with the finding of parabasal cells "post-natal" cells in smears, there was no evidence of fetal trauma. Thus Ezes conception proved wrong, and the finding of parabasal cells in smears of prolonged pregnancy is not related to fetal trauma.

At the same time it was shown that there is a danger of hypoxia for the fetus, in cases of persistence longer than physiological limits permit of the "clearly at term" cytological pattern. This hypothesis was borne out in 11 cases of either damaged or stillborn infants.

Determination of the term of labor on the basis of the vaginal smear and its diagnostic importance in prolonged pregnancy. Židovsky, Jan: Akuserstvo i ginekologija. **2:** 37-45, 1960.

ACCORDING to data of manifold investigations of the vaginal smear cytology in 427 pregnant women, four types of vaginal smears are distinguished: 1st type—during late pregnancy, labor sets in 10 days or later; 2nd type—labor takes place in 4-7 days; 3rd type—in 1-5 days, and the 4th type—labor occurs in the same day or within the next 3 days.

If, in prolonged pregnancy, the 1st or 2nd type of smear is found, expectant therapy can be resorted to, inasmuch as there is no hazard for the fetus of hypoxic syndrome development. When the 3rd or 4th type of vaginal smear is revealed with the gestation uninterrupted for over five days, immediate enforcement of labor is necessary, in view of the fact that the fetus is in danger due to postponed labor.

Rapid identification of malignant cells in vaginal smears by cytoplasmic fluorescence. Elevitch, Franklin R. and Joel G. Brunson: Surg., Gynec. & Obst. **112:** 3, 1961.

VAGINAL aspirations and cervical scrapings from 641 unselected gynecological and obstetrical patients were screened for uterine malignant neoplasms, with acridine orange fluorescence by an observer untrained in cytology. Presence of intense red-orange cytoplasmic fluorescene, which reflects an increased amount of ribonucleic acid, was considered presumptive evidence of malignancy. In seven (44%) of 18 such smears called AO positive, malignancy was actually present. Most AO false positives (six of 11) were from patients who had been irradiated for carcinoma of the cervix. Present evidence indicates that the increased RNA in a cervical squamous cell carcinoma occurs in rapidly proliferating cells. Reasons for false negatives are discussed. By restricting AO fluorescence screening to detection of early carcinomas which contain normally growing cells, it is conceivable that greater accuracy will be obtained.

The technic is rapid and simple. It appears to be valuable in screening a normal population in that there is a low percentage of false positive results. It does not appear to be of value in screening post-radiation patients for recurrence of malignant neoplasms. In this study 70 per cent (seven of ten) of primary uterine malignant tumors are identified properly by AO fluorescence of cytoplasm.

The effect of certain chemical and physical agents on the radiation sensitivity of mouse tumors. Modlin, R. K. and J. McL. Morris: Cancer. **14:** 117, 1961.

BY a technic employing transplanted mouse tumors, the following observations were made on radiation sensitivity:

1. Intravenous administration of glutathione prior to radiation therapy increased radiation resistance and significantly lowered tumor remission rate.

2. This effect could be reversed by concomitant testosterone administration.

3. Testosterone administered alone produced

a more favorable response initially but this was not significant after 40 days.

4. Increase temperature (to an average of 40 C) appeared to accelerate radiation response of the tumors and produced marked increase reaction of the normal tissue without improving the ultimate remission rate. There was a suggestion that cold might delay radiation response.

5. Anoxia produced by a tourniquet appeared to decrease radiation sensitivity of both tumor and normal tissue.

6. Surgical incision into the tumor done prior to radiation therapy improved the remission rate of the tumors. Incision three days after radiation therapy had somewhat less effect, but there was no evidence that surgery interfered with tumor radiation response.

Progression of experimental cervical dysplasia in the mouse. Christopherson, W. M. and W. Broghamer, Jr.: Cancer. **14:** 201, 1961.

THE application of a carcinogen to the cervix of a mouse results in a gradual increase in cellular atypia until invasive carcinoma results in the death of the animal. Once the stage of dysplasia is reached, invasive carcinoma eventually develops despite withholding further applications of the carcinogen.

A critical study of cervical biopsies including serial sectioning. Christopherson, W. M. and J. E. Parker: Cancer. **14:** 213, 1961.

ONE HUNDRED cervical biopsy specimens, 24 conization specimens and five complete cervices were examined by serial step sectioning. The specimens were from women with abnormal cervical cell studies. Fourteen of the 129 specimens showed a greater degree of abnormality in the step sections than they did in the original routine sections. The factors involved in obtaining suitable specimens and the indications for serial sectioning are discussed.

Cancer of the cervix—a biometric approach to etiology. Stern, E. and W. J. Dixon: Cancer. **14:** 153, 1961.

A STATISTICAL survey of a preliminary selection of eight variables was undertaken in a group of women that included those with no evidence of cancer and those with newly discovered atypical hyperplasia and preinvasive and invasive cancer of the cervix. The method of multiple regression analysis made possible the examination of all variables simultaneously.

The variable, marital events, was found to be important in separating those with atypical hyperplasia — preinvasive and invasive cancer from the controls. The term, as employed here, indicates multiple changes in marital status and implies emotional and endocrine instability. The study has shown this finding to be of sufficient importance that further research is indicated to clarify its relation to the etiology of cancer of the cervix.

In this study the variable, currently married, was found to be a comparatively greater value in separating individuals with precancerous lesions from those with cancer.

Adequate interpretation of these findings must await further inquiry into what accounts for the observed association of being currently not married with the presence of atypical hyperplasia.

Such factors as race, religious affiliation, nationality, and circumcision, thought to be important when considered singly, because of their obvious association with special groups of women in whom cervix cancer rates were exceptionally high or low, were found to be of secondary significance in this study.

Different variables are found to be important in the studies using both normal individuals and those with atypical hyperplasia and preinvasive and invasive cancer when compared to those studies in which normal individuals are omitted. This suggests that different factors are associated with the initiation of cancer of the cervix than are associated with the promotion of this disease from one stage to another. When normal individuals are included, the variables, marital events, and Jewish women, are of primary importance. When normal individuals are not included, the variable, age (perhaps indicating the effect of the passage of time), is the dominant factor.

Office examination of fresh cancer cells by interference phase microscopy. Hirst, Donald V.: Am. J. Obst. & Gynec. **81:** 138, 1961.

EIGHT photomicrographs from an interference phase microscope are presented of fresh cancer cells which show new and striking differences from cancer cells examined after fixing and staining. Fresh cancer cells are apparently fragile, lyse easily, and may be destroyed by fixing solutions.

Changing ratio of cervical to corpus carcinoma. Helwig, Ferdinand C.: Am. J. Obst. & Gynec. **81:** 277, 1961.

1. A 32-year survey of 1,608 cases of cancer of the cervix and endometrium is presented. For

the first two decades of this study, the ratio of cervical to fundal cancer was roughly 2:1. This figure was quite different from many reports in the literature for this same period, which ranged from 10:1 to 5:1, as well as from a survey of similar cases from a large local municipal charity hospital.

2. The possible reasons for this discrepancy, such as geographic location and race and economic status, were mentioned.

3. Following 1951, a decrease in published figures from 10:1 to 3:1 of cervical cancers to corpus cancer was noted, and some of the possible reasons for this change are discussed.

4. In our survey, a significant drop in ratio of invasive cervical cancer to endometrial cancer from 2:1 to 1:1 was observed since 1948.

5. The influence of a steadily increasing employment of routine Papanicolaou stains on all cervices with biopsy or conization of all patients with positive smears is discussed, and the ratio of *in situ* carcinoma of the cervix to carcinoma of the fundus was found to be almost 1:1. Statistically, this figure, when combined with a 1:1 ratio for invasive cervical cancer, might suggest that carcinoma *in situ* is a genuine, preinvasive stage of invasive cancer.

6. A quite definite decrease in advanced cervical and endometrial cancer in our material is pointed out.

Results in the management of preclinical carcinoma of the cervix. Latour, J.P.A.: Am. J. Obst. & Gynec. **81:** 511, 1961.

As a result of the analysis of this group of 274 patients with preclinical cancer of the cervix, we are prompted to make the following conclusions:

1. Routine cytologic screening has detected a relatively large number of preclinical squamous cancers of the cervix but appears to have missed the early adenocarcinomas.

2. Both preinvasive and invasive preclinical carcinomas are recognized. These should be separated and identified for the sake of uniform statistical reporting. In this respect there appears to be a need for modification of the International Classification, as suggested by Kottmeier.

3. Both the average age of the patients and the response to treatment indicate that early invasive preclinical cancer is closer kin to carcinoma *in situ* than to clinical carcinoma. The clinical appearance of the cervix would seem to be an important factor in prognosis.

4. Preclinical cancer of both varieties responds well to a variety of treatments. Management should be individualized after close cooperation between the experienced clinician and the pathologist. There appears to be very infrequent need for radiation therapy or radical surgical procedures.

5. The definitive treatment of choice appears to be total hysterectomy with a wide vaginal cuff and conservation of ovarian function when practicable.

6. The degree of therapy inherent in complete circumferential biopsy procedures appears to offer the young woman desirous of further pregnancies adequate security, and we recommend this course provided the patient and her husband understand the risks involved and accept perpetual periodic cytologic screening as the only method of protection.

Cervical cancer and sex activity: cancer of the cervix in prison population. Pereyra, A. J.: Obst. & Gynec. **17:** 154-159, 1961.

CONCLUSIONS: 1. A survey for cancer of the cervix conducted among the prisoners of the California Institution for Women in 1957-58 revealed this disease to be six times more prevalent in this group than among comparable non-prison groups.

2. This disparity in rate was found to be limited to cancer of the cervix and did not apply to other forms of cancer encountered among these institutionalized women.

3. Local tissue injuries incident to childbirth, inflammatory condition, erosion, pelvic disease, and histories of venereal infection were very common among these women, but a causal relationship of these factors to cervical cancer was not established.

4. The one common characteristic present in this group which can be integrated into the findings of other investigators is the variegated and promiscuous sexual activity of these women.

5. This study supports the concept of cervical cancer as an epidemiological entity and upholds the hypothesis of an exogenous agent, possibly a virus transmitted by sexual intercourse, as being involved in the production of this disease.

6. The value of the Papanicolaou cervical smear in the early diagnosis of cancer of the cervix and the importance of using this procedure at an early age and more frequently in promiscuous women are emphasized by the results of this study.

7. The association of sexual activity with

cancer of the cervix does not exclude other factors that may initiate or aggravate the disruption of the chromatin autonomy with resultant mutations and malignancy.

Early diagnosis of cervix-carcinoma. Soost, H. J.: Ärzt. Praxis i. B. 1: 70, 1959.

It is claimed that any changes on the ectocervix and vaginal bleedings that are suspicious of carcinoma, a cytologic smear ought to be taken even by a general practitioner and should be sent to a central laboratory for examination. The technic of taking a smear is demonstrated by a series of pictures. According to statistical reviews the success of cytology in looking for tracings of gynecologic cancer is presented. (Author's Abstract.)

The prognostic value of vaginal cytology in pregnancy. Nesbitt, R. E. L., Rogelio Garcia, and Doris S. Rome: Obst. & Gynec. 17: 2-8, 1961.

The vaginal smear for cytohormonal diagnosis should become an integral part of the physicians' armamentarium. It is a practical screening technic for determining the end effect of the combined vaginal action of estrogens and progesterone. By the same token, it is a highly accurate method for forecasting pregnancy outcome and for testing the efficacy of the therapeutic regime.

The data presented here would seem to justify the following conclusions pertinent to clinical practice.

1. When the vaginal smear persistently has a karyopyknotic index of less than 20, the patient has a less than 1 per cent chance of aborting from endocrine related causes.

2. If the smear is abnormal, the likelihood of aborting is related directly to the percentage of karyopyknotic cells appearing in the vaginal smear.

3. Only 8.9 per cent of those who ultimately aborted in a series of 504 unselected patients did so in the presence of a good smear, and only 1.3 per cent of the patients with a good smear ultimately aborted.

4. Only 3.0 per cent of the patients who carried to term did so in the presence of a consistently poor smear, and several of these individuals later developed difficulties due to placenta abnormalities.

5. Patients who have had a previous fetal loss have slightly increased likelihood of aborting, but the factor of smear type is of far greater importance in forecasting pregnancy outcome.

6. When the karyopyknotic index is high in patients with vaginal bleeding and lower abdominal crampy pain, the likelihood of abortion is great.

7. The vaginal smear indicates an increased progesterone effect as a result of hormone therapy only when the prognosis for the pregnancy is good.

8. Patients who have a reversible progestational defect, as reflected by a change in smear type in response to hormone therapy, usually have a normal chorionic gonadotrophin titer. When the titer is low the outlook for the pregnancy is grave, regardless of cytologic findings.

9. Absence of a good cytologic response to therapy, especially the occurrence of increased vaginal cornification following estrogen treatment, constitutes an extremely poor prognosis.

10. Endocrine factors would not seem to be causal in patients who threaten to abort in spite of a good smear. The outlook for the pregnancy is generally good, however, and it is not necessary to institute hormonal treatment. The outcome will be uninfluenced by such therapy.

11. Hormone therapy administered after the onset of symptoms on a purely empirical basis will not appreciably influence the overall salvage rates compared with those achieved by nonspecific therapeutic measures. A great majority of these patients will demonstrate in their smears a high index of karyopyknosis; the placental deterioration has often reached an irreversible stage.

12. It is reasonable to administer hormone treatment to those patients who demonstrate abnormal vaginal smears. The response of the smear to such therapy should determine the benefit derived from it and the wisdom of continuing it.

13. The procurement and study of vaginal smears obtained weekly during the critical months of early pregnancy will provide an effective means of testing the efficacy of therapeutic hormone regimes and of forecasting the ultimate outcome of pregnancy.

The so-called perinuclear halo. De Girolami, Ettore: Obst. & Gynec. 17: 175, 1961.

The term perinuclear halo is used to define a morphologic change usually present in the squamous cells of the uterine cervix during certain types of inflammation. Using the Papamilitiades-Shorr stain and nigrosine, it was

observed that the clear area is not around the nucleus but is intranuclear. In fact, the nucleus is the chromosome frame, the clear area is the nucleoplasm, and the membrane is the nuclear membrane. The presence of chromatin material in the clear area and in accumulations along the nuclear membrane adds support to the postulation that the morphologic change is intra- and not extranuclear, as had been generally considered. The karyotoxic action of the exotoxins (or endotoxins) is so great in this type of inflammation that the nuclear pathologic manifestations are more evident than those of the cytoplasm. As in nuclear damage after irradiation, the precocious and rapid contraction of the chromatin frame, not followed by progressive loss of the nucleoplasm and progressive contraction of the nuclear membrane, produces the picture of the intranuclear clear area.

Importance of a cytologic examination of breast secretion in cases of glandular dysplasia or cancer (importanza dell 'esame citocariologico della secrezione mammaria in alcune condizioni displasiche e neoplastiche della ghiandola). Conte, Diego and G. Barbasetti di Prun: Attualita di Ostetricia e Ginecologia. **5, 6:** 1135, 1959.

A CYTOLOGIC examination was made of the breast discharge of 102 women. The results are discussed, emphasizing the value of this method, which the authors advocate as a routine examination.

Cytological observations in threatened and retained abortion, and in abortive puerperium (osservazioni colpocitologiche nella minaccia di aborto, nel puerperio abortivo, e nell 'aborto ritenuto). Berlingieri, Domenico and C. Labate: Attualita di Ostetricia e Ginecologia. **5, 6:** 1157, 1959.

AN INVESTIGATION of the vaginal cytology, based on the determination of cytologic Karyopyknotic Indices, was carried out on 55 women with threatened abortion, retained abortion, or abortive puerperium. In threatened abortion there was a remarkable increase in both indices: when the threat was past the values of the indices returned to nearly normal.

The indices values were low in abortive puerperium, corresponding to the reduced stratification of the vaginal epithelium, while in retained abortion there wasn't sufficient cytologic evidence to make a definite diagnosis of the interruption of pregnancy. Finally the author discusses extensively the pathogenesis of the cytologic changes.

Cytological aspect of tuberculosis of the female genital tract (aspetti citologici della tuberculosi dell'apparato genitale femminile). Tortora, M.: Atti Societa Italiana di Ostetricia e Ginecologia. **47:** 1060, 1959 (Congresso di Bari.)

GIANT CELLS were observed in endometrial aspirate smears from four cases of endometrial tuberculosis, diagnosed by biopsy. Some of these cells showed peripheral disposition or peculiar configuration of the nuclei; the author believes these to be Langhans cells desquamating from the tubercular lesion.

Study of the cervical cytology of threatened abortion in the first trimester of pregnancy (studio colpocitologico nelle minacce di aborto del primo trimestre di gravidanza). Pasquinucci C.: Annali di Ostetricia e Ginecologia. **81:** 971, 1959.

THE AUTHOR examined the vaginal smears of 50 women with threatened abortion in the first three months of pregnancy. Despite wide variations and aspect of typical aspect in vaginal cytology during this period, the author found it to be of value as a guide in diagnosis and prognosis, and as an indication of the efficiency of therapy adopted.

A synthetic progestational agent (17-ethynyl-19-nor-testosterone) was used as therapy in these cases, but no hormonal change was evident in the vaginal smears. The author advances a probable explanation for this.

Cystic glandular hyperplasia in the atrophic (senile) endometrium cyto - hormonal evaluation, histochemistry, and pathologic considerations (l'iperplasia ghiandolare cistica dell'endometrio nell 'eta' senile indagini cito-ormoniche ed istochimiche e considerazioni patogenetiche). Berlingieri, Domenico: Archivio di Ostetricia e Ginecologia. **65:** 3, 1960.

THE AUTHOR gives a brief critical review of the works on cystic glandular hyperplasia in the atrophic (senile) endometrium. In ten selected cases he determines the rate of urinary excretion of estrogens and the Karypyknotic Index in the cervical smear, and discusses their relation to each other. He emphasizes the difference among end-organ responses in senile women, and maintains that histopathology may be attributed in some cases to a heightened responsiveness of the endometrium to slight but prolonged estrogenic stimulus.

Contribution to the understanding of cervical and vaginal response to hormones (contributo alla conoscenza della recettivita' cervico-vaginale agli ormoni vaginali). Berlingieri, Domenico and F. Nobili: Archivio di Ostetricia e Ginecologia. **65:** 4, 1960.

THE AUTHORS studied the response of the cervix and the vagina to parenterally administered estrogens and androgens in 45 women of different ages, 26 of whom were menopausal or castrates. This was done by means of the cervical mucus crystallization test and the cytological index according to Moracci.

The vaginal mucosa was shown to be more sensitive than the cervical mucosa to the hormones used. The local conditions of responsiveness and the mode of action of the hormones are discussed.

Aspects of endometrial cytology in the normal menstrual cycle (aspetti di citologia endometriale nel ciclo mestruale normale). Centonze, M.: Monitore Ostetrico e Ginecologico di Endocrinologia e del Metabolismo. **1:** , 1959.

ENDOMETRIAL cytology of healthy women was investigated by intra-uterine aspirate at various stages of the menstrual cycle. The smears were stained by the Papanicolaou method: In many cases control biopsies were made.

The author describes cyclic alterations in the stromal and columnar cells. The morphological and endocrinological value of the technics used is stressed.

Value of "imprint" cytology for the rapid intra-operational evaluation of new growths. Wierdis, T. and T. Bacay: Minerva Medica Ginecologia. **12-13:** 647-651, 1960.

ONE HUNDRED "imprint" specimens from various sites, each with a histological section from the same location as control, were examined by the authors who then evaluated imprint cytology as a means of differentiating between malignant and non-malignant tumors. It was found that cellular morphology may be evaluated accurately by this technic, and because of the ease and rapidity with which it may be used, imprint cytology can be a great diagnostic aid to the surgeon. Imprint specimens have the advantages of being almost completely free of blood and other connective elements, permitting greater accuracy of cytologic interpretation in many cases. The diagnostic limitations are that neither the type of tumor, nor its exact location may be deduced from an imprint specimen.

Four intra-uterine cytological aspirations in the first stages of labor, prematurely and at term (quadri citologici daaspirazione endouterina in prossimita' dell travaglio di parto, prematuro e a termina). Centonze, M. and Pasquale de Salvia: Monitore Ostetrico e Ginecologica di Endocrinologia e del Metabolismo. **1:** , 1959.

SMEARS were made from material aspirated from the intra-uterine cavities of women in the early stages of labor and stained by the Papanicolaou method. The morphological patterns are described.

Exfoliative cytology of chorionepithelioma and hydatiform mole (la citologia esfoliativa nel corionepitelioma e nella mola vesicocare). Centonze, M.: Quaderni di Clinica Ostetrica e Ginecologica. **15:** 9, 1960.

THE WRITER describes vaginal smears and uterine smears obtained by aspirations from cases of chorionepithelioma and hydatiform mole. The cytologic patterns are discussed from the standpoints of diagnostic hormonal evaluation and cellular morphology.

Aspects of cytodiagnosis of cancer of the uterine cervix (aspetti di citodiagnostica nel cancro del collo dell'utero). Centonze, M. and Pasquale de Salvia: Quaderni di Clinica Ostetrica e Ginecologia. **15:** 9, 1960.

THE AUTHORS discuss some of the more important aspects of diagnosis of uterine cancer by exfoliative cytology, and stress the importance of extending cytologic examination to a greater number of women. The morphological characteristics of malignant cells and the classification of smears are discussed, with emphasis on the particular importance of the Papanicolaou Class III smears.

These writers feel that it is not possible to differentiate cytologically between invasive and non-invasive cancer. The more common causes of cytological diagnostic and prognostic errors are examined.

The German Society of Applied Cytology

The Constitution of the German Society of Applied Cytology:

Deutsche Gesellschaft für Angewandte Cytologie

Satzung:

1. **Name:** Der Name der Vereinigung ist: *Deutsche Gesellschaft für angewandte Cytologie.*

2. **Sitz:** Der Sitz der Vereinigung ist Heidelberg. Die Vereinigung soll in das Vereinsregister eingetragen werden.

3. **Zweck und Ziele:**
   a. Die Vereinigung wird gegründet als nationale wissenschaftliche Organisation für Cytologen und an der Cytologie Interessierte. Sie ist in corpore Mitglied der Europ. Gesellschaft für klin.u.soziale Cytologie. Sie soll in sich und als Mitglied der europ. Fachorganisation die Zusammenarbeit unter den Personen fördern, die Cytologie treiben, an der weiteren wissenschaftlichen u.praktischen Ausbreitung und Anwendung cytologischer Untersuchungsmethoden interessiert sind oder die Ergebnisse der Methode in ihrem Arbeitsbereich verwerten.
   b. Sie will:
   die *Früherkennung des Krebses* durch den Einsatz cytologischer Untersuchungsmethoden fördern, die Bevölkerung über die Möglichkeiten einer Früherkennung aufklären, die *wissenschaftliche Grundlagenforschung der Cytologie* auf den verschiedenen Fachgebieten unterstützen und durch Meinungsaustausch mit Vertretern der Randgebiete fördern, dem *Unterricht in klinischer Cytologie* und der Ausbildung des Nachwuchses an cytologisch tätigen Ärzten und techn. Hilfskräften einen Standard geben und *Richtlinien für eine international anerkannte Ausbildungsnorm* ausarbeiten, für die *Vereinheitlichung von Ausbildung,* Nomenklatur, cytologischer T e c h n i k Sorge zu tragen, den *Austausch* von Ärtzen, Studenten und techn. Hilfskräften anregen und durchführen vermittels ihrer gewählten Vertreter als *Berater bei nationalen Körperschaften* und bei der Europ. Gesellschaft für klin.u.soziale Cytologie tätig sein, soweit Probleme der Krebserkennung mittels cytologischer Untersuchungen oder andere Arbeitsbereiche der klin.u.sozialen Cytologie in Frage kommen.

4. **Mittel zur Erreichung der Ziele:**
   a. Als das offizielle Organ der Gesellschaft gilt die Acta Cytologica der Internationalen Akademie für gynäkologische Cytologie (Chicago, Editorial Office 5841 South Maryland Ave, Chicago 37, Ill. USA), solange nicht die Europ. Gesellschaft von sich aus ein eigenes Organ schafft.
   b. Rundschreiben in zwangloser Folge sollen die Mitglieder über Stand der Arbeit, Arbeitspläne, Neuerscheinungen auf cytologischem Gebiet informieren.
   c. Im Rahmen der Europ. Organisation wird einmal jährlich ein Treffen in einer Stadt eines europ. Landes stattfinden, um die Cytologie betreffenden Probleme zu erörtern und einen Hauptarbeitsplan für die folgenden 2 Jahre aufzustellen.
   d. Im Rahmen der Europ. Gesellschaft sollen alle 2 Jahre die Arbeitsergebnisse und Jahresarbeiten in einem Band gedruckt und verbreitet werden.

5. **Mitglieder der Gesellschaft:**
   a. *Ehrenmitglieder:* gehören der Gesellschaft auf Grund ihres wissenschaftlichen Rufes oder ihrer Verdienste für die Förderung der Cytologie an.
   b. *Cytologisch tätige Mitglieder:*
   Ärzte, die aktiv Cytologie treiben und im Jahr mindestens 1000 Präparate zu beurteilen haben,
   Ärzte, die als Leiter cytologischer Untersuchungsstellen tätig sind,
   Ärzte, die Cytologie lehren.
   c. *Mitglieder:*
   Ärzte aller Fachrichtungen, die an der Anwendung cytologischer Untersuchungsmethoden interessiert sind,
   Ärzte und Wissenschaftler der Grenzgebiete, die an der wissenschaftlichen Anwendung der Cytologie beteiligt u. interessiert sind.
   d. *Technische Mitglieder:* wissenschaftliche Hilfskräfte und techn. Assistenten, die an der techn. Durchführung der cyto-

logischen Untersuchungsmethoden beteiligt sind oder im Rahmen dieser Untersuchungen statistische Aufgaben erfüllen.

e. *Fördernde Mitglieder,* die durch ihren Einfluss in der Öffentlichkeit als einzelne oder juristische Person oder durch materielle und ideelle Förderung die Ziele der Gesellschaft unterstützen.

6. Organisation der Gesellschaft:

a. Organe der Gesellschaft sind:
   (1) der Vorstand
   (2) die Mitgliederversammlung.

b. Die Geschäfte der Gesellschaft werden vom Vorstand wahrgenommen. Dieser besteht aus:
   Präsident
   Sekretär (Stellvertreter)
   Schatzmeister
   Vorstand im Sinne des § 26 BGS ist der Präsident und sein Stellvertreter.

c. Die Wahl des Vorstandes erfolgt durch die Mitgliederversammlung alle 2 Jahre. Während dieses Zeitraumes und bis zur Neuwahl des neuen Vorstandes führen die Vorstandsmitglieder die Geschäfte der Gesellschaft.

d. Der Vorstand ist gehalten, einen Beirat zu bilden, bestehend aus
   3 Mitgliedern unter 5 b (Cytologen)
   3 Mitgliedern unter 5 c (Mitglieder)
   2 Mitgliedern unter 5 d (techn. Mitglieder)
   2 Mitgliedern unter 5 e (fördernde Mitglieder)
   Dieser Beirat hat beratende Stimme.

e. Der Vorstand ist ermächtigt, für besondere Fragen Fachkommitees zu ernennen.

7. Schirmherrschaft:

Wie in jedem Mitgliedsland der europ. Gesellschaft wird ein Kommittee der Schirmherrschaft gebildet, das sich aus Mitgliedern unter 5 e zusammensetzt und etwa 5 Personen umfassen soll. Diese Personen sollen d e m europ. Zusammenschluss wohlwolend gegenüberstehen und durch ihren Einfluss in der Öffentlichkeit die Ziele der Gesellschaft zu einer europäischen Zusammenarbeit unterstützen.

8. Ernennung von Ehrenmitgliedern:

Ehrenmitglieder werden vom Vorstand nach Anhören des Beirates ernannt.

9. Eintritt und Austritt:

Zum Eintritt ist berechtigt jede Person, welche die Voraussetzungen der Mitgliedschaft unter 5) erfüllt. Die Aufnahme erfolgt unter Befürwortung von 2 Mitgliedern, die entweder Gründungsmitglieder sind oder eine zweijährige Mitgliedschaft aufweisen. Der Austritt erfolgt auf eigenen Entschluss durch schriftliche Erklärung an den Vorstand.

Der Ausschluss eines Mitgliedes kann erfolgen

(a) bei Verlust der bürgerlichen Ehrenrechte

(b) auf Grund eines Zweidrittelmehrheitsbeschlusses des Vorstandes und des Beirates. Berufung gegen diesen Beschluss ist zulässig. Sie wird der Mitgliederversammlung vorgetragen, die mit Zweidrittelmehrheit entscheidet.

Antrag auf Ausschluss kann durch jedes Mitglied beim Vorstand schriftlich gestellt werden.

10. Beiträge:

Die Höhe des Beitrages wird von der Vollversammlung nach Ablauf von höchstens 2 Jahren nach Gründung der Gesellschaft festgelegt. Die Hälfte der Beiträge soll zur Förderung der europ. Zusammenarbeit verwandt werden.

11. Mitgliederversammlung:

Mindestens alle 2 Jahre hat eine Mitgliederversammlung stattzufinden. Ihr obliegt die Entgegennahme des Jahresberichtes und der Jahresabrechnung, der Vorstandsschaft, weiter die Wahl der Vorstandsmitglieder, die Entlastung der Vorstandsmitglieder, die Festsetzung des Jahresbeitrages für Mitglieder und Beschlussfassung über Satzungsänderungen oder Auflösung.

Die Mitgliederversammlung ist vom Vorstand schriftlich unter Angabe der Tagesordnung und unter Einhaltung einer Frist von mindestens 2 Wochen einzuberufen.

Die Mitgliederversammlung ist ohne Rücksicht auf die Zahl der Erschienenen beschlussfähig. Die Mitgliederversammlung fasst ihre Beschlüsse mit einfacher Mehrheit. Zu Satzungsänderungen ist jedoch eine Dreiviertelmehrheit der Erschienenen, zur Auflösung eine Vierfünftelmehrheit erforderlich.

12. Die Beurkundung der Beschlüsse:

Die in der Vorstandssitzung oder Mitgliederversammlung gefassten Beschlüsse sind schriftlich niederzulegen und vom Versammlungsleiter und dem Protokollführer zu unterzeichnen.

13. Auflösung der Vereinigung:
Die Auflösung kann nur in einer Mitgliederversammlung beschlossen werden. Hierzu ist Vierfünftelstimmenmehrheit der Erschienenen erforderlich. Sofern die Mitgliederversammlung keine besonderen Liquidatoren bestellt, sind der Präsident und sein Stellvertreter zu Liquidatoren zu ernennen. Die Liquidatoren haben die laufenden Geschäfte abzuwickeln, das vorhandene Vermögen flüssig zu machen und nach dem Ausgleich das Restvermögen dem Zentralausschuss für Krebsbekämpfung zur Verfügung zu stellen.

Sociedade Brasileira De Citologia

Report on Scientific Activity during 1960

During the year 1960 the Sociedade Brasileira de Citologia conducted four ordinary sessions, a symposium and a round table conference.

First Session, March 1960 (16th Session of the Society): A paper on "Significação de vacuolos em celulas" was presented by Dr. Grimaldo de Carvalho; a paper on "Identificação de celulas neoplasicas no sangue circulante por intermedio do microscopio de fluorescencia" was given by Dr. Pimenta de Mello.

Second Session, June 1960 (17th Session of the Society): A paper by Dr. C. A. Ferreira on "Citodiagnostico do adcnocarcinoma uterino" was presented.

Third Session, August 1960 (18th Session of the Society): Dr. Roberto Silveira presented a paper on "O valor real da colpocitologia."

Fourth Session, November 1960 in Sao Paulo (19th Session of the Society): With the Pathology Department "Associação Paulista de Medicina," with the following papers: "Aspectos gerais da citologia clinica, finalidades e metodos" by Dr. Fernando T. Mendes; "Citologia ginecologica," by Dr. Antonio Cardoso de Almeida; "Citologia dos liquidos de derrame," by Dr. Paulo E. Mello; "Citologia gastrica," by Dr. Fernando Mendes and João Martins; "Citologia pulmonar," by Dr. Luiz Gonzaga Saraiva; "Citologia urinaria," by Dr. Melchior Sawaya; "Citologia otorrinolaringologica" by Dr. Terezinha F. Lorenzi.

Round Table Conference, October 1960 at the occasion of the VI Congresso Brasileiro de Ginecologia Obstetricia: Two subjects, "Cytology at the end pregnancy" and "Possibilities of cytologic diagnosis of Carcinoma *in situ*" were presented and discussed by: Drs. Jean de Brux, C. A. Ferreira, Nilo P. Luz and Mario B. Kamnitzer.

II Simposio Brasileiro de Citologia, October 6 and 7, 1960 consisted of four scientific sessions with the following presentations: Dr. Jean A. de Brux: "La metaplasie active indifferenciée immature"; Dr. Guillermo Terzano: "Citologia exfoliativa en epitelios anomalos"; Dr. M. B. Kamnitzer: "Susa-Gomori, metodo rapido de coloração," "Classificação do ciclo gravidico-puerperal," "Colpocitologia na determinação do parto," "Colpocitologia no abortamento"; Dr. Carlos Marigo: "Citologia de contato na punçao biopsia do figado; estudo comparativo"; Dra. Regina Calmon e Dr. Alberto Lima: "Relatorio das atividades do Serviço de Citologia do Hospital A. Maltez durante 5 anos de funcionamento"; Dr. Wulmar Bastos: "Citologia no ato cirurgico," "Citodiagnostico do cancer nos aparelhos digestivo, respiratorio, genitourinario"; Drs. Ilka Menezes, Hildegard Stoltz e Grimaldo de Carvalho: "Avaliação do SR em pacientes tratadas por cancer no I.G.U.B."; Dr. Nilo P. Luz: "O diagnostico citologico do termo da gravidez"; Dr. J. Rezende, Belfort Aguiar e J. M. Barcelos: "Diagnostico da amniorrexis; citologia, determinação das gorduras, *p*H, cristalisação," "Amniorrexis prematura, diagnostico e conduta"; Dr. João P. Martins: "A colpocitologia no pre-parto"; Dr. Noé Zamel: "Citologia gastrica, experiencia pessoal"; Dr. Abraão Carpilowski: "Um novo metodo para colheita de material em citologia exfoliativa"; Drs. Aloysio A. Sá e Ivan Lemgruber: "Observaçoes sobre o estudo colpocitologico de 100 ciclos, no I.A.P.B."; Dr. Ivan Lemgruber: "Colpocitologia e dosagens hormonais," estudo em 50 casos"; Dras. Helia Maldonado e C. A. Ferreira: "Melhoria do diagnostico citologico do adenocarninoma do corpo uterino pelo aspirado intracavitario"; Dr. Vitorio Valeri e colab.: "Citologia endometrial, considerações sobre 52 casos de colheita intracavitaria."

Correspondence

Assessment of Radiosensitivity by Means of Exfoliative Cytology (SR)

To the Editors: The method of assessment of radiosensitivity known as SR was used as a means of evaluation in our patients soon after it was introduced by Drs. Graham. Reference is made to the article by B. Cornelius Hopman entitled "Can the cytological or the histological response to radiation be influenced by medication or other factors?" published in *Acta Cytologica*, **3**: 428, 1959.

Hopman writes: "In a recent meeting of the Inter-Society Cytology Council in New York (Nov. 1958), Grace Herman, as a result of the study of 200 cancer cases, came to the conclusion that SR is a nonspecific response closely allied to the endocrine status of the patient rather than a specific response to carcinoma. Fennel using fluorescent antibody technic, found that no specific antibody was detected in given vaginal cells, as related to sensitization response."

Independently, we evaluated the vaginal smears of 140 women not affected by cervical cancer to determine whether SR was evident in noncancerous smears. Our principal criterion for SR was densely vacuolated cytoplasm.

We examined 159 vaginal smears from the 140 women. Of this total, 30 were in the age group from 16 to 41, and suffered from current hormonal disturbance; 30 were young girls from two days to seven years of age; 30 were menopausal women, of ages 51 to 81; 50 were postpartum cases, the smears being taken in the period from the first day to the fourth month after delivery. In this last group the 19 women who showed SR were re-examined cytologically after their first menstrual period. It may be in the following table that we found SR in nonmalignant cases.

	Number of cases	High SR	SR%
Hormonal disturbances	30	1	3.0
Children	30	7	23.3
Climacteric women	30	9	30.0
Post partum	50	19	38.0

It is evident from these results that high SR was found in cases in which one would expect low estrogenic excretion. This expected correlation has been confirmed by G. Herman, H. Hughes, and S. Gusberg. Children, menopausal women, and postpartum women showed a substantially higher SR than women with normal or high estrogenic cytological patterns. Of the 19 postpartum cases with high SR, none showed high SR after the first menstrual period.

Although this is merely a small sample, we may cautiously infer that SR is not an independent criterion, but varies in conformity with the hormonal status of the woman.

Dr. Otakar Nyklíček, *Director,*
Department of Obstetrics and Gynecology
Náchod, Czechoslovakia

Normal Processes in Cervical and Vaginal Epithelia and Their Implications in Malignant Growth

To the Editors: Reference is made to the Symposium on Possible or Probable Pre-Malignant Lesions:

In considering various physiological activities of the cervical and vaginal epithelia, one has to realize, first of all, that these epithelia throughout life undergo *renewal of their constituent cells.* Continuously, cells are shed from the epithelia, as this is evident in any sample of vaginal and cervical aspirate, and the cells are replaced by new ones formed by mitosis. This process of cell renewal is a normal physiological process peculiar of a large number of epithelia, such as those of the alimentary tract,[1] the epidermis,[2, 3] the epithelia of trachea and bronchi,[4] and many others. No definite figures of the rate at which cell renewal in cervical and vaginal epithelia occurs are yet available. Preliminary investigations on rats, using the colchicine method,[5, 6] seem to indicate, however, that these epithelia are completely renewed within less than a week. If this is the case, the cell renewal times of vaginal and cervical epithelia are within the range of those of stratified squamous epithelia of other regions (esophagus, 8.8 days; oral epithelia four to five days[1]). Their renewal is thus faster than that of the epidermis (19 days[2, 3]). Cell renewal times of columnar epithelia show a greater range, from 1.5 days (small intestine[1]) to 48 days (bronchus). It is true that in normal epithelia, with the exception, possibly, of that of the intestine, mitotic figures are rarely seen. However, it has to be

realized that at any instant of time only very few cell divisions are necessary for rapid renewal of an epithelium.

Moreover, it has been shown[7] that after death a large percentage of cells which had entered division shortly before death, still continue mitosis to the resting stage. However, no new mitoses are initiated after death. Thus, in autopsy material, which was not fixed immediately, the mitotic rates of tissues will be lower than they actually were during the life of the individual.

The cells which by mitosis supply new ones to replace those cells lost from the surface by desquamation, frequently are of rather undifferentiated type; in the case of stratified epithelia, they are usually located in the basal portion of the epithelia. Their primary significance is mitotic division rather than such metabolic activities as secretion. The only recognizable activity other than division is that, in some epithelia, they also partake in the formation of a reticular basement membrane.

In the cervical epithelium, which generally is considered to be simple columnar, there is inserted between the columnar cells and the basement membrane a more or less distinct row of flattened, rather undifferentiated cells. It is these "reserve cells" that, by mitotic division, supply new columnar secretory and ciliated cells, to replace those which continuously desquamate from the epithelium.

In the vaginal epithelium, divisions of basal cells keep the number of cells of the epithelium constant or in a "steady state," by renewing the numerous cells which are continuously shed from the surface in the form of intermediate and superficial cells. Of course, the thickness of the epithelium varies with the stages of the menstrual and estrous cycles which possibly affect directly the mitotic rates of the basal cells.

Following division, some of the "reserve cells" of the cervical epithelium and some of the basal cells of the vaginal epithelium remain at the base of the epithelia to supply further cells by subsequent division, whereas others differentiate into mucous secreting or ciliated cells and intermediate and superficial cells respectively.

Fully differentiated epithelial cells, in the adult, have to a large extent lost their ability to divide. Thus, normally, intermediate and superficial cells, and actively secreting cells, usually do not divide. The activity of differentiated cells is directed toward secretion, absorption, protection, etc. rather than toward multiplication. Therefore, it is probable that it is mainly the undifferentiated cells, whether they are "reserve cells," basal cells, or cells of similar category, that initiate abnormal cellular reactions, such as hyperplasia, dysplasia and malignancy.

Recent work indicates that there are some primarily quantitative differences in cytochemistry between malignant and nonmalignant cells. However, in many respects, the chemistry of malignant cells seems either to be very similar to basal or "reserve" cells, or, with the exception of the nucleic acids, most so far discovered differences in cell chemistry cannot readily be employed, with the present technics, for the use in routine diagnosis. However, recognition of such changes may supply valuable additional information.

It would be expected that malignant cells, similar to the basal cells and "reserve cells," contain little or no *glycogen* and *mucopolysaccharides*. Malignant cells are primarily engaged in proliferation rather than in secretion or other activities performed by normal epithelial cells. The increase in malignant cells of *acid phosphatase* which plays a role in glycogen metabolism, may either be a consequence of above, or may be independent and linked up with the increase in nucleic acids, and thus in the long run, with increased protein metabolism of proliferating cells.

An increase of *desoxyribonucleic acid* (DNA) in nuclei may be of various nature. Firstly, there may be a true increase in DNA, i.e., polyploidy, as this is characteristic of many malignant cells. Secondly, some 18 to 24 hours before cell division, the DNA proteins double in the nucleus (chemical prophase) to supply eventually a complete set of chromosomes to each daughter cell. Usually, in normal tissues, this physiological increase is not readily recognized morphologically; it could possibly be at least partially responsible for the hyperchromatism of some cells in actively proliferating tissues. And thirdly, because the amount of DNA is constant in all normal nuclei within a species, irrespective of the kind of tissue,[8] small nuclei with the same amount of desoxyribonucleic acid packed within a smaller volume will appear more basophil than nuclei of larger volume. This becomes obvious if one compares, for example, the nuclei of lymphocytes with those of large neurons. Thus, also pyknotic nuclei, which had not yet lost larger amounts of DNA, may appear more basophil and this may sometimes be merely optical rather than being caused by an actual increase in DNA. The above considerations should be kept in mind when deter-

mining morphologically an increase of DNA in cells.

A very significant cytochemical change in hyperplasia, dysplasia, and malignancy, which is valuable for routine diagnosis, is the often pronounced increase in *ribonucleic acid* (RNA). As it is well known, RNA is closely linked with protein synthesis of the cell, and there may be a direct relationship between the amount of RNA present in a cell and its activity in protein synthesis. Thus, secreting cells which produce such substances as, for example, muco-proteins, are often characterized by a moderate content of RNA, which forms with proteins such structures as the ergastoplasm. Especially proliferating cells, which synthetize proteins for the formation by mitosis of daughter cells, are rich in RNA. Therefore, basal cells, "reserve cells," and others, supplying new cells in the course of cell renewal, usually contain larger amounts of RNA than do their differentiated descendents. This is especially apparent in stratified squamous epithelia where the amount of RNA gradually decreases as the cells approach the superficial layers. It is less obvious in the columnar cervical epithelium, where many of the cells are secretory and, therefore, always contain moderate amounts of RNA. However, if cells proliferate at a rate faster than normal, as this is the case, for example, in erosions, the cells also show larger amounts of RNA.

In proliferating malignant tumors, whose mitotic rates usually exceed by far those of normal tissues, the cells contain large amounts of cytoplasmic RNA. Many cells remain undifferentiated and therefore capable of mitosis. They thus seem to continue protein synthesis in preparation for subsequent division.

An increase in RNA content, may it be moderate in some normal "active" cells, such as in erosions, or large as in malignant cells, usually is not readily recognized with routine staining methods, which primarily demonstrate morphological rather than cytochemical changes.

Because cytochemical changes, such as those of RNA content, must precede morphological changes which are largely a consequence of the latter, early recognition of such changes may possibly lead to the earlier diagnosis of malignancy, or of premalignant changes.

The simplest tool for the routine study of changes in nucleic acid content in cells is the fluorescence method, using acridine orange.[9] This technic quantitatively demonstrates the nucleic acids in cells, and especially RNA. Cells with little or no RNA (e.g., superficial squamous cells), appear in green fluorescence. Those with small amounts of RNA (e.g., macrophages) fluoresce brown, whereas cells with moderate RNA content (e.g., basal, parabasal, and endocervical cells) show reddish brown fluorescence. In contrast, malignant cells, which have a large RNA content, appear in bright orange through flaming red fluorescence. On the basis of their striking fluorescence, malignant cells give an orange-red warning signal recognized even with low scanning powers. In addition, the (fluorescence microscope) technic reveals morphological features with almost similar clarity as do routine technics, and nuclei, which fluoresce green (DNA), are easily studied for morphological changes. Also an increase in proliferation rate of non-malignant cells is readily recognized with the fluorescence technic. Thus, in erosions, endocervical cells and parabasal cells exhibit an increased reddish fluorescence; the otherwise normal morphology of these cells distinguishes them clearly from suspicious cell elements. Likewise, "active" macrophages, for example, of the respiratory system and in body fluids, proliferating more actively presumably owing to chronic irritations, appear with increased fluorescence as do also more actively proliferating mesothelial cells, cells from the respiratory epithelium, and others.

Thus to date, in addition to morphological criteria, cytochemical changes in ribonucleic acid content appear to be the most significant for cancer diagnosis, because they can be readily recognized.

FELIX D. BERTALANFFY, PH.D.
Associate Professor of Anatomy, University of Manitoba, Winnipeg 3, Manitoba, Canada

AND

LUDWIG VON BERTALANFFY, PH.D.
The Menninger Foundation, Topeka, Kansas, U.S.A.

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To the Editors: Reference is made to the papers by Herovici, entitled "Histo- and Cytochemistry of Basal and Parabasal Cells" and "Histo- and Cytochemistry of Intermediate Cells," in *Acta Cytologica*, 4: 51-53, 54-55, 1960. May I refer especially to his *Conclusion* in the first paper where he states: "The basal vaginal layer is characterized (1) by its richness in nucleic acids (DNA and RNA), in alkaline phosphatases and in potassium, and (2) by the absence of glycogen, polysaccharides and sulphydrilic protides. It does not seem to be affected by cyclic modifications" and also to his *Closing Remarks* after the discussion of the second paper where the author states: ". . . I have never found changes in the DNA-content in the intermediate layer of the vaginal epithelium."

Haour (*ibid.* page 55) discussing the paper of Herovici states concerning the author's conclusion: "We agree with Herovici's findings concerning the absence of any cyclic variations of the DNA content of intermediate vaginal cells during the length of the menstrual cycle. . . ."

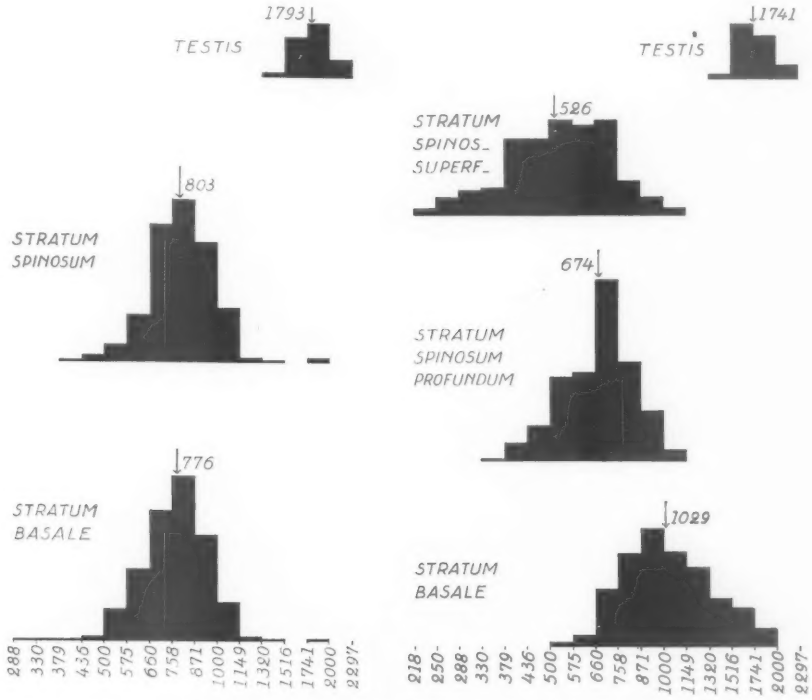

FIG. 1. (left) Histograms showing the nuclear DNA content of the vaginal epithelium of the mouse in diestrus. FIG. 2. Histograms showing the nuclear DNA content of the vaginal epithelium of the mouse in estrus.

If I understand these authors correctly they favor the opinion that the concentration of nuclear DNA of exfoliated basal, parabasal or intermediate squamous cells show no cyclic variations.

As far as women are concerned this may be true though I would doubt it. As a matter of fact we have clearly demonstrated by means of a histophotometric device (Thiery, M., Archives de Biologie **71**: 389-404, 1960) that the nuclear DNA content of the vaginal epithelium in mice varies during the estrous cycle: it increases during the stages of active growth and cell anabolism (proestrus and estrus) and decreases during metestrus. A definite correlation exists between the DNA content of the nucleus and the physiological activity of the cell. Keratinization of the vaginal epithelium seems to be accompanied by progressive reduction of the DNA content of the nucleus.

MICHEL THIERY, M.D.
Laboratoire d'Anatomie humaine et d'Anatomie comparée (Dir.: Prof. Dr. J. Fautrez) et Section gynécologique du Centre anti-cancereux (Dir.: Prof. Dr. F. DeRom), State University Ghent, Ghent, Belgium.

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The Ninth Annual Meeting of the Inter-Society Cytology Council, Inc. will be held at the Hotel Peabody, Memphis, Tennessee, Thursday through Saturday, November 2-4, 1961. The proposed amendments to the Constitution and By-laws sent to all voting members in December 1960 will be acted upon at the Annual Business Meeting, November 3, 1961.

The Tenth Annual Meeting of the *American Society of Cytology* (formerly Inter-Society Cytology Council) will be held in St. Louis, Missouri, November 8-10, 1962.

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